GeneMarker®

The Biologist Friendly Software

User Manual

SOFTGENETICS®

Software PowerTools for Genetic Analysis

www.softgenetics.com

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Chapter 1 Installing GeneMarker

Chapter 1 Installing GeneMarker
Computer System Requirements
Validation Version
Local Version
Network Version
Questions

Computer System Requirements

GeneMarker software has been tested and validated for various computer systems. The minimum system requirements are:

Windows® PC
OS: Windows® XP, Vista, Windows® 7
Processor: Pentium® III, 1 GHz CPU
RAM: 512MB
Available hard disk space: 20GB

Intel® Powered Macintosh®
Parallels® desktop for Mac (Mac OS/virtual machine dependent) or Apple™ Boot Camp or VMware® Fusion (Mac OS/virtual machine dependent)
RAM: 2GB
Available hard disk space: 20GB

Installation of GeneMarker is not supported on Linux or UNIX-based operating systems.

GeneMarker will only recognize PC file formats. To convert Macintosh file formats to PC file formats, please download the ABI PRISM® 3100 Genetic Analyzer Conversion Utilities to convert Mac files to PC files at: **http://www.appliedbiosystems.com/support/software/3100/conversion.cfm**

Validation Version

The validation or trial version of GeneMarker can be installed on as many computers as you wish. The trial period expires 35 days after installation of the software.

Installation

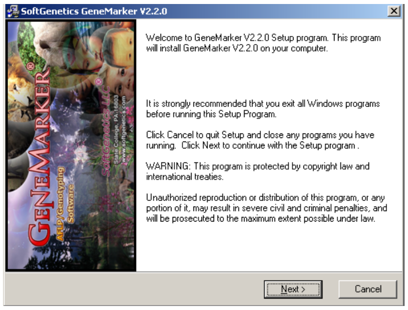
1. Insert the *SoftGenetics CD* into the CD-ROM drive. If your computer is not set to automatically open a CD, navigate to the optical or CD-ROM drive on the computer and open the directory.
2. Double-click the GeneMarker Setup executable file (EXE)
3. The *Installation Wizard* will launch
4. Click the **Next** button in the *Welcome* window
5. Read the *SoftGenetics End User License Agreement* and click the **I Agree** button in the *Read Me File* window
6. Select "Install GeneMarker (Recommended)" in the *Select Program* window and click Next
7. Click **Next** in the *Destination Location* window to install GeneMarker in the default folder. Click the **Browse** button to choose a different installation directory

NOTE: The default *Destination Location* for the GeneMarker program is C:\ProgramFiles\SoftGenetics\GeneMarker\"version number"

8. Click **Next** in the *Select Program Manager Group* window to accept the default *Program Manager Group*

NOTE: Changing the *Program Manager Group* default may affect program operability. It is recommended to accept the default.

9. Click **Next** in the *Start Installation* window to install GeneMarker
10. Click **Finish** in the *Installation Complete* window
11. The *Installation Wizard* will close
12. Eject the *SoftGenetics CD*
13. Launch GeneMarker by double-clicking the **GeneMarker** desktop icon OR open the **Start** menu and navigate to *SoftGenetics → GeneMarker, the version that was just installed → GeneMarker program*
14. The *Configure* window will appear. Click **Run Validation** to launch the software
15. If the **Run Validation** button is grayed-out this indicates the 35-day trial period has expired.



Welcome to GeneMarker V2.2.0 Setup program. This program will install GeneMarker V2.2.0 on your computer.

It is strongly recommended that you exit all Windows programs before running this Setup Program.

Click Cancel to quit Setup and close any programs you have running. Click Next to continue with the Setup program .

WARNING: This program is protected by copyright law and international treaties.

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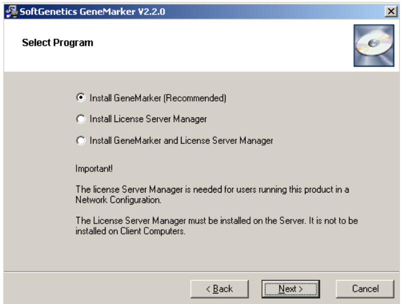
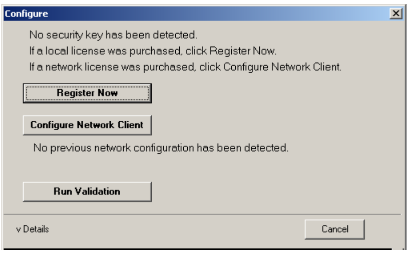


● Install GeneMarker (Recommended)
○ Install License Server Manager
○ Install GeneMarker and License Server Manager

Important!

The license Server Manager is needed for users running this product in a Network Configuration.

The License Server Manager must be installed on the Server. It is not to be installed on Client Computers.



No security key has been detected.
If a local license was purchased, click Register Now.
If a network license was purchased, click Configure Network Client.

No previous network configuration has been detected.

Local-licensing Option

GeneMarker v2.00 and above supports text-based registration for the local-licensing option—no USB device, dongle, key or hardware is required. This text-based registration ID is registered to one specific PC. If the license needs to be transferred to a different PC, registration for that one license/PC must be inactivated first before the software will be registered to the new PC.

Installation

1. Insert the *SoftGenetics CD* into the optical or CD-ROM drive. If your computer is not set to automatically open a CD, navigate to the optical or CD-ROM drive on the computer and open the directory.
2. Double-click the GeneMarker Setup executable file (EXE)
3. The *Installation Wizard* will launch
4. Click the **Next** button in the *Welcome* window
5. Read the *SoftGenetics End User License Agreement* and click the **I Agree** button in the *Read Me File* window
6. Select "Install GeneMarker (Recommended)" in the *Select Program* window and click Next
7. Click **Next** in the *Destination Location* window to install GeneMarker in the default folder. Click the **Browse** button to choose a different installation directory

NOTE: The default *Destination Location* for the GeneMarker program is C:\ProgramFiles\SoftGenetics\GeneMarker\ver#

8. Click **Next** in the *Select Program Manager Group* window to accept the default *Program Manager Group*

NOTE: Changing the *Program Manager Group* default may affect program operability. It is recommended to accept the default.

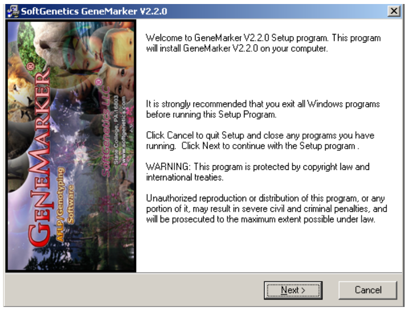
9. Click **Next** in the *Start Installation* window to install GeneMarker
10. Click **Finish** in the *Installation Complete* window
11. The *Installation Wizard* will close
12. Eject the *SoftGenetics CD*
13. Launch GeneMarker by double-clicking the **GeneMarker** desktop icon OR open the **Start** menu and navigate to *SoftGenetics* → *GeneMarker, the version that was just installed* → *GeneMarker program*
14. The *Configure/Registration* window will appear. Click **Register Now** to register the local license
15. Click **Register Local Text-based Key** from the *Choose Registration Method* dialog box

Registration

1. The *Register Local Text-based Key* window appears
2. If the computer GeneMarker is being installed on has an internet connection, select **Online Registration**. If the computer does not have an internet connection or is connected to a proxy server, select **Offline Registration**.

Online Registration

A. Locate the **Account** and **Password** on the *SoftGenetics CD*.
B. Enter your *Account*, *Password,* and *e-mail address* information in the appropriate fields
C. The *Request Code* information is automatically generated by GeneMarker
D. Click **Register**


SoftGenetics GeneMarker V2.2.0
GENEMARKER®
AFLP/Genotyping Software
SoftGenetics LLC®
State College, PA 16803
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Next >
Cancel

Welcome to GeneMarker V2.2.0 Setup program. This program will install GeneMarker V2.2.0 on your computer.

It is strongly recommended that you exit all Windows programs before running this Setup Program.

Click Cancel to quit Setup and close any programs you have running. Click Next to continue with the Setup program .

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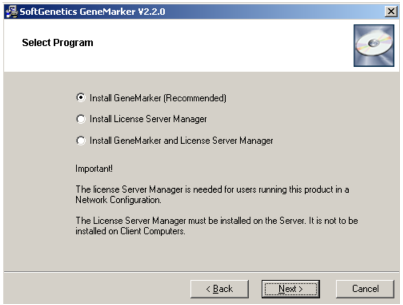
SoftGenetics GeneMarker V2.2.0
Select Program
< Back
Next >
Cancel

● Install GeneMarker (Recommended)
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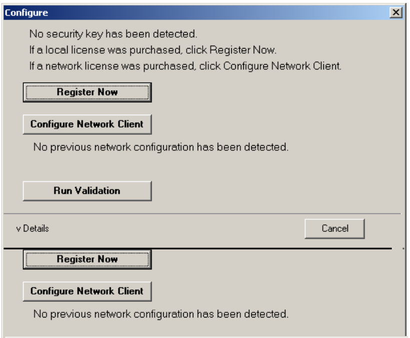
Important!

The license Server Manager is needed for users running this product in a Network Configuration.

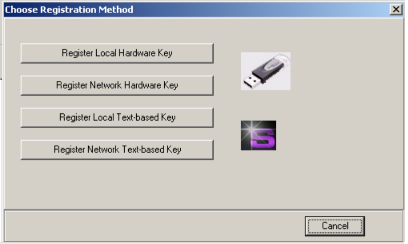
The License Server Manager must be installed on the Server. It is not to be installed on Client Computers.

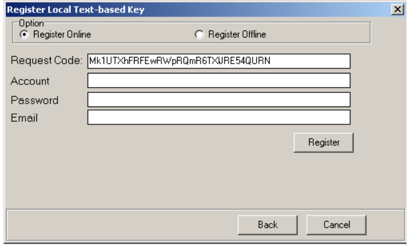

Configure
Register Now
Configure Network Client
Run Validation
v Details
Cancel
Register Now
Configure Network Client

No security key has been detected.
If a local license was purchased, click Register Now.
If a network license was purchased, click Configure Network Client.

No previous network configuration has been detected.

No previous network configuration has been detected.


Choose Registration Method
Register Local Hardware Key
Register Network Hardware Key
Register Local Text-based Key
Register Network Text-based Key
Cancel


Register Local Text-based Key
Option
Register
Back
Cancel

● Register Online ○ Register Offline

Request Code: MkIUTXhFRFEwRWpRQmR6TXlJRE54QURN
Account
Password
Email

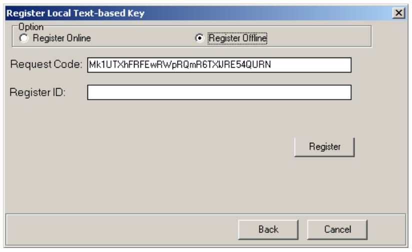
E. Your software will be registered automatically. A confirmation e-mail will be sent to you once registration is complete.

NOTE: Some characters can commonly be misread. If you get an error trying to register, check for number “1” and lower case letter “L” or number “0” and upper case letter “O” confusion.

F. Launch GeneMarker and begin analysis

Offline Registration

A. Copy and paste the entire *Request Code* string and type your *Account* and *Password* information from the *SoftGenetics CD* into the body of an e-mail
B. Send the email to tech_support@softgenetics.com
C. The *Registration ID* will be sent to you (via email) within one business day
D. Copy and paste the *Registration ID* from the e-mail into the *Registration ID* field
E. Click **Register**
F. Launch GeneMarker and begin analysis



● Register Offline
Request Code: Mk1UTXhFRFEwRWpRQmR6TXJRE54QURN
Register ID:

Upgrade

There are two local-licensing options available for GeneMarker v2.0 and above: USB-device required and text-based.

The legacy local-licensing option of GeneMarker available for previous versions (v1.97 and earlier) of the software is supported for v2.0 and above. This option requires a USB device (dongle-, key-, hardware-based). This option allows installation on many computers. However, the associated USB device must be inserted into the USB port of the computer to operate the program.

If you wish to exchange hardware-based licensing for text-based licensing please contact **info@softgenetics.com**.

Installing Over the Previous Version

If you choose to install the new version of GeneMarker over the previous version, you will need to choose the same directory for installation. Several of the old files will be replaced with newer files. Other files that are not present during installation but are created during analyses or by the user will remain in the folder and can easily be recognized by the new version of GeneMarker. Please make a backup copy of the target directory before installing the newer software.

Installing into a New Directory

If you choose to install the newer version in a different location, be sure to specify a unique directory name or Program Manager Group for the upgrade to prevent overwriting any previous versions of GeneMarker. Several files created by the users or created during analyses conducted by the previous version of GeneMarker will not be recognized by the new version of GeneMarker unless they can be found in the directory of installation. If you intend for the new version to recognize these files, then you will need to copy them from the older version’s installation folder and paste them in the folder containing the new version of GeneMarker.

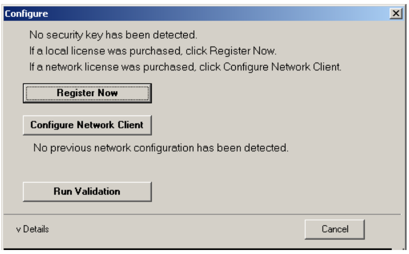
Some of the more common customized GeneMarker files are: GeneDB.mdb, GeneMarker.mdb, codis.ini, CommentsTemplate.ini, ExpTemplates.ini, Panel folder and SizeStd folder.

Upgrade Procedure — Text-based

1. Before proceeding, prepare a backup copy of the previous version of GeneMarker (recommended)
2. Double-click the GeneMarker executable file (EXE) on the *SoftGenetics Upgrade CD*.
3. Proceed through the *Installation Wizard* as described in the *Installation* section above
4. Once the *Installation Wizard* is complete, launch GeneMarker by double-clicking the new **GeneMarker** desktop icon *OR* open the **Start** menu and navigate to *SoftGenetics* → *GeneMarker, the version that was just installed* → *GeneMarker* program
5. The *Configure/Registration* window appears. Click **Register Now** to register the local license
6. Click **Register Local Text-based Key** from the *Choose Registration Method* dialog box
7. Proceed through the *Registration* steps as described in the *Registration* section above
8. Launch GeneMarker and begin analysis

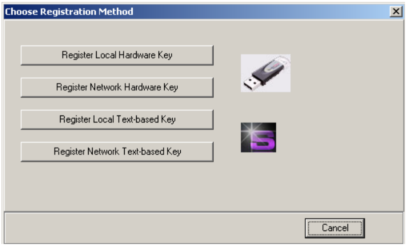
Upgrade Procedure — Hardware-based

1. Before proceeding, prepare a backup copy of the previous version of GeneMarker (recommended)
2. Ensure the GeneMarker USB key is inserted into the computer's USB port
3. Double-click the GeneMarker executable file (EXE) on the *SoftGenetics Upgrade CD*.
4. Proceed through the *Installation Wizard* as described in the *Installation* section above
5. Once the *Installation Wizard* is complete, launch GeneMarker by double-clicking the new **GeneMarker** desktop icon OR open the **Start** menu and navigate to *SoftGenetics → GeneMarker, the version that was just installed → GeneMarker* program
6. The *Configure/Registration* window appears. Click **Register Now** to register the local license
7. Click **Register Local Hardware Key** from the *Choose Registration Method* dialog box. The *Register Local Hardware Key* window appears
8. If the computer GeneMarker is being installed on has an internet connection, select **Online Registration**. If the computer does not have an internet connection or is connected to a proxy server, select **Offline Registration**.
9. Proceed through the *Registration* steps as described in the *Online Registration* or *Offline Registration* section above
10. Launch GeneMarker and begin analysis



No security key has been detected.
If a local license was purchased, click Register Now.
If a network license was purchased, click Configure Network Client.

No previous network configuration has been detected.


Choose Registration Method
Register Local Hardware Key
Register Network Hardware Key
Register Local Text-based Key
Register Network Text-based Key
Cancel

Network-licensing Option

The network-licensing version of GeneMarker can be installed on any computer in a network configuration. SoftGenetics uses the License Server Manager (LSM) to control the number of concurrent users accessing the network-licensing option of GeneMarker v2.00 (and above). LSM uses text-based registration—no hardware is required. Both software components are installed from the same EXE. The computer where License Server Manager program is installed is considered the "Server" computer. Computers on the network other than the Server are called "Client" computers.

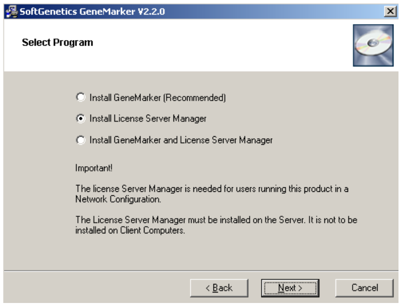
Installing License Server Manager will require restarting the system to complete installation. Please save all work and close all applications before installing LSM.

Install License Server Manager

1. Insert the *SoftGenetics CD* into the optical or CD-ROM drive. If your computer is not set to automatically open a CD, navigate to the optical or CD-ROM drive on the computer and open the directory.
2. Double-click the GeneMarker Setup executable file (EXE)
3. The *Installation Wizard* will launch
4. Click the **Next** button in the *Welcome* window
5. Read the *SoftGenetics End User License Agreement* and click the **I Agree** button in the *Read Me File* window
6. Select "Install License Server Manager" in the *Select Program* window and click Next
7. Click **Next** in the *Destination Location* window, **Next** in the *Select Program Manager Group* window, and Next in the *Start Installation* window to enter the LSM installation wizard
8. Click the **Next** button in the *Welcome* window
9. Read the *SoftGenetics End User License Agreement* and click the **I Agree** button in the *Read Me File* window
10. Click **Next** in the *Destination Location* window to install LSM in the default folder. Click the **Browse** button to choose a different installation directory

NOTE: The default *Destination Location* for the License Server Manager program is C:\ProgramFiles\SoftGenetics\License Server

11. Click **Next** in the *Start Installation* window to install License Server Manager

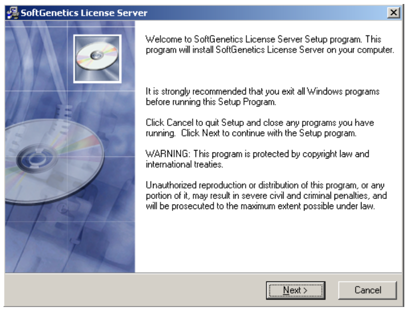


○ Install GeneMarker (Recommended)
● Install License Server Manager
○ Install GeneMarker and License Server Manager

Important!

The license Server Manager is needed for users running this product in a Network Configuration.

The License Server Manager must be installed on the Server. It is not to be installed on Client Computers.



Welcome to SoftGenetics License Server Setup program. This program will install SoftGenetics License Server on your computer.

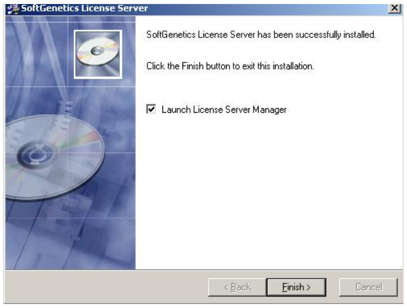
It is strongly recommended that you exit all Windows programs before running this Setup Program.

Click Cancel to quit Setup and close any programs you have running. Click Next to continue with the Setup program.

WARNING: This program is protected by copyright law and international treaties.

Unauthorized reproduction or distribution of this program, or any portion of it, may result in severe civil and criminal penalties, and will be prosecuted to the maximum extent possible under law.

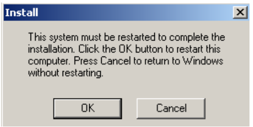
12. Select the Launch License Server Manager option and click **Finish**
13. Click **OK** in the *Install* window to restart the system.
14. The *Installation Wizard* will close and the system will restart
15. Eject the *SoftGenetics CD*


SoftGenetics License Server
< Back
Finish >
Cancel

SoftGenetics License Server has been successfully installed.

Click the Finish button to exit this installation.

☑ Launch License Server Manager


Install
OK
Cancel

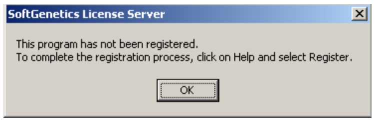
This system must be restarted to complete the installation. Click the OK button to restart this computer. Press Cancel to return to Windows without restarting.

Register License Server Manager for GeneMarker Usage

1. Open License Server from the System or Icon Tray by clicking the LSM icon

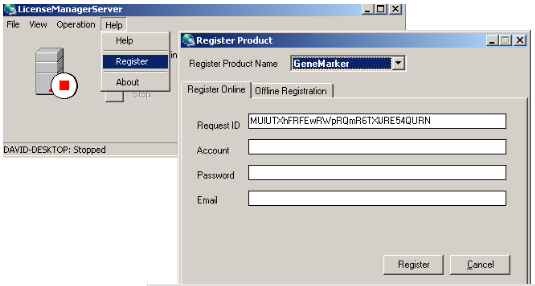
Note: A red star indicates the License server is not running. The icon with a white star indicates the License Server is running properly.

2. Click **OK** in the dialog box to proceed with registering License Server from the License Server Manager console.


SoftGenetics License Server
OK

This program has not been registered.
To complete the registration process, click on Help and select Register.

3. Select Register from the Help menu to activate the Register Product window
4. Select GeneMarker from the Register Product Name drop-down menu.
5. SelecIf the computer License Server is being installed on has an internet connection, select **Online Registration**. If the computer does not have an internet connection or is connected to a proxy server, select **Offline Registration**.


LicenseManagerServer
File
View
Operation
Help
Help
Register
About
Stop
Register Product
Register Online
Offline Registration
Register
Cancel

DAVID-DESKTOP: Stopped

Register Product Name: GeneMarker

Request ID: MUIUTXhFRFEwRWpRQmR6TXJRE54QURN

Account

Password

Email

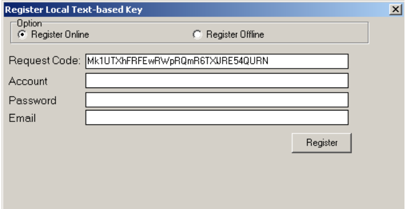
Online Registration

A. Locate the **Account** and **Password** on the *SoftGenetics CD*
B. Enter your *Account*, *Password*, and *e-mail address* information in the appropriate fields
C. The *Request Code* information is automatically generated by License Server
D. Click **Register**

E. Your software will be registered automatically. A confirmation e-mail will be sent to you once registration is complete.

NOTE: Some characters can commonly be misread. If you get an error trying to register, check for number "1" and lower case letter "L" or number "0" and upper case letter "O" confusion.

F. Restart License Server to apply the registration information.


Register Local Text-based Key
Register
Back
Cancel

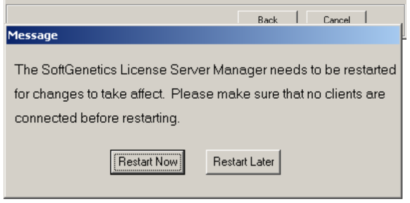
Option
● Register Online ○ Register Offline

Request Code: Mk1UTXhFRFEwRWpRQmR6TXJRE54QURN

Account

Password

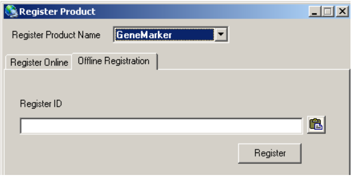
Email


Message
Restart Now
Restart Later

The SoftGenetics License Server Manager needs to be restarted for changes to take affect. Please make sure that no clients are connected before restarting.

Offline Registration

G. Copy and paste the entire *Request Code* string and type your *Account* and *Password* information from the *SoftGenetics CD* into the body of an e-mail
H. Send the email to tech_support@softgenetics.com
I. The *Register ID* will be sent to you (via email) within one business day
J. Copy and paste the *Registration ID* from the e-mail into the *Register ID* field of the Offline Registration tab
K. Click **Register**

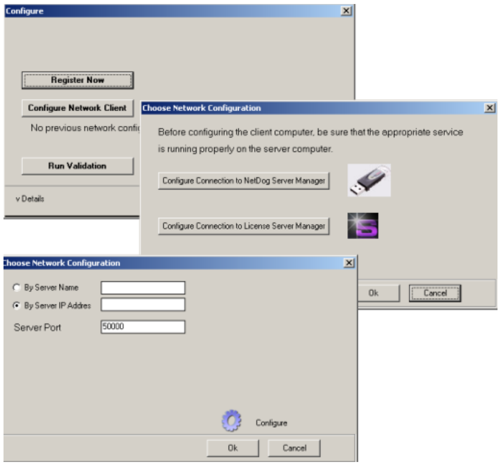

Register Product
Register Online
Offline Registration
Register

Register Product Name: GeneMarker

Register ID

Install GeneMarker software on the client computer

1. Proceed with installing GeneMarker software on the client computer as described in the "Local-licensing Option, Installation" section above until the *Configure/Registration* window appears
2. Click **Configure Network Client** to configure the client software to contact License Server Manager
3. Click **Configure Connection to License Server Manager** from the *Choose Network Configuration* dialog box
4. Input Server Name or Server IP Address
5. Click Configure and GeneMarker software will automatically open if connection is properly established and a license is available.


Configure
Register Now
Configure Network Client
Run Validation
v Details
Choose Network Configuration
Configure Connection to NetDog Server Manager
Configure Connection to License Server Manager
Ok
Cancel
Choose Network Configuration
Configure
Ok
Cancel

No previous network confi

Before configuring the client computer, be sure that the appropriate service is running properly on the server computer.

○ By Server Name
● By Server IP Addres
Server Port 50000

Upgrade of License Server Manager

Activate the License Server Manager console

Proceed with step 3 of "Register License Server Manager for GeneMarker Usage" section above

Upgrade of GeneMarker software on client computer

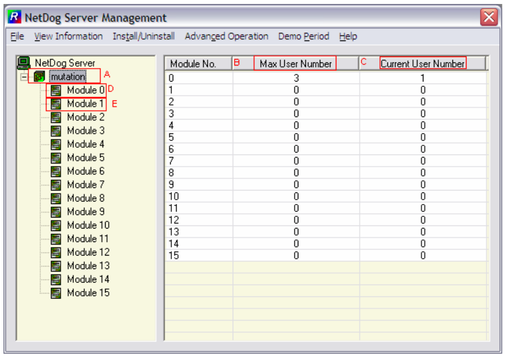
Install GeneMarker software on the client computer by following the procedure in the "Install GeneMarker software on the client computer" section above.

If the network configuration has not changed the software should activate without configuring the IP address of License Server.

Install NetDog Server Management

The following are instructions for installing the NetDog Server application. See *Local Version Installation* section above for instructions on installing the GeneMarker program.

1. Insert the NetDog Key (USB/parallel hardware key) into the computer that will run the NetDog Server software. The computer may be any client computer on your network that runs Microsoft Windows.
2. Launch the setup file of NetDog Server (/NetDog Server Setup/setup.exe) and install the server.
3. After installation, NetDog Server starts automatically.


NetDog Server Management
File
View Information
Install/Uninstall
Advanced Operation
Demo Period
Help
NetDog Server
mutation
A
Module 0
D
Module 1
E
Module 2
Module 3
Module 4
Module 5
Module 6
Module 7
Module 8
Module 9
Module 10
Module 11
Module 12
Module 13
Module 14
Module 15
B
C

Module No.	Max User Number	Current User Number
0	3	1
1	0	0
2	0	0
3	0	0
4	0	0
5	0	0
6	0	0
7	0	0
8	0	0
9	0	0
10	0	0
11	0	0
12	0	0
13	0	0
14	0	0
15	0	0

4. Double-click the R icon on the task bar to open up the *NetDogServer Management* application.
    - A. The section in the figure that A is referring to shows that the NetDog Server is able to detect the NetDog. If it is unable to detect the NetDog, it will display mutation (please refer to the section about installing the NetDog key to correct this).
    - B. Column B indicates the maximum number of concurrent users that are allowed to access the software.
    - C. Column C indicates the number of current users running the software.
    - D. The "Module 0" row indicates programs being used in the network version of Mutation Surveyor (Not applicable if you do not have Mutation Surveyor).
    - E. The "Module 1" row indicates programs being used in the network version of GeneMarker.
5. After installing the NetDog Server Program, please configure any firewalls on the server to allow the following default ports:

    TCP/IP: 4587

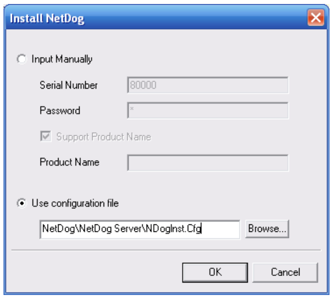
UDP/IP: 4587
IPX: 17799

If other ports are preferred, please launch the *NetDog Server Management* console and make the necessary changes.

Install the NetDog Key

In the toolbar, there is an option to *Install* or *Uninstall*, which allows you to delete or re-install the NetDog.

1. Delete any existing "Mutation" NetDog Key before beginning the installation.
2. Click **Install NetDog** and the *Install NetDog* box will appear

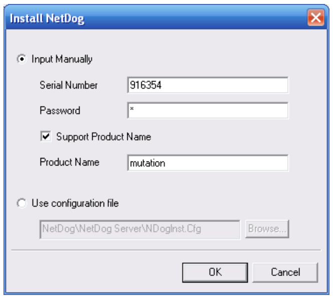

Install NetDog
Input Manually
Serial Number
Password
Support Product Name
Product Name
Browse...
OK
Cancel

○ Input Manually
Serial Number: 80000
Password: *
☑ Support Product Name
● Use configuration file
NetDog\NetDog Server\NDogInst.Cfg

3. Import the NDogINst.cfg file, which is located in the same directory as the NetDog Server setup package.
4. The NetDog Server can also be installed manually. Select the *Input Manually* option and type in the Serial Number and Product name.

NOTE: **Do not change the Password**.

5. Click OK


Install NetDog
Input Manually
Serial Number
Password
Support Product Name
Product Name
Use configuration file
Browse...
OK
Cancel

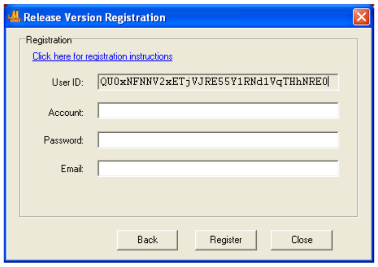
● Input Manually
Serial Number: 916354
Password: *
☑ Support Product Name
Product Name: mutation
○ Use configuration file
NetDog\NetDog Server\NDogInst.Cfg

Registration

1. The *Network Version Registration* window appears
2. If the computer GeneMarker is being installed on has an internet connection, select **Online Registration**. If the computer does not have an internet connection, select **Offline Registration**.
3. Click **Next**

Online Registration

A. Locate the **Account** and **Password** on the *SoftGenetics CD*.
B. Enter your *E-mail Address*, *Account*, and *Password* information in the appropriate fields
C. Click **Register**
D. The USB key will automatically load the User ID information into the registration field.
E. Your software will be registered automatically. A confirmation e-mail will be sent to you once registration is complete.


Release Version Registration
Registration
Click here for registration instructions
User ID:
Account:
Password:
Email:
Back
Register
Close

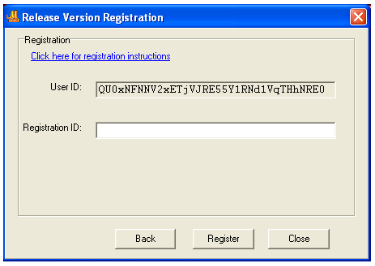
User ID: QU0xNFNNV2xETjVJRE55Y1RNd1VqTHhNRE0

NOTE: Some characters can commonly be misread. If you get an error trying to register, check for number "1" and lower case letter "L" or number "0" and upper case letter "O" confusion.

F. Launch GeneMarker and begin analysis

Offline Registration

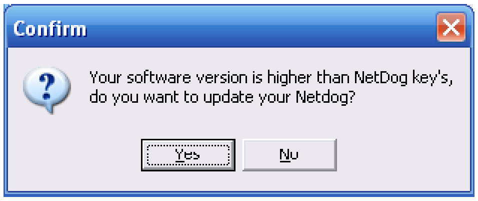
A. Copy and paste the entire *User ID* string and type your *Account* and *Password* information into the body of an e-mail.
B. Send the email to tech_support@softgenetics.com.
C. The *Registration ID* will be sent to you (via email) within one business day.
D. Copy and paste the *Registration ID* from the e-mail into the *Registration ID* field.
E. Click **Register**
F. Launch GeneMarker and begin analysis


Release Version Registration
Registration
Click here for registration instructions
User ID:
Registration ID:
Back
Register
Close

User ID: QU0xNFNNV2xETjVJRE55Y1RNd1VqTHhNRE0

Upgrade

The upgrade version of GeneMarker Network should be installed on the server – the computer running NetDog Server – as well as all client computers.



Your software version is higher than NetDog key's, do you want to update your Netdog?

1. First install upgrade network version on the server computer. You will be prompted to configure the network (enter server name or IP address).
2. After installing and configuring the upgrade you should update NetDog on the server computer. To do this, launch the *NetDog Upgrade* tool. NetDog Update can be accessed by going to *Start menu* → *All Programs* → *SoftGenetics* → *GeneMarker (Network)* → *NetDogUpdate*. It is located in the same directory as the GeneMarker program.
3. The *Confirm* dialog box will appear if the versions of GeneMarker and NetDog are not consistent.
4. Click **Yes** and proceed to software registration.

Additional User Licenses

If you are interested in purchasing additional user licenses, please launch the upgrade tool (**NetDogUpdate.exe**) that is located in the GeneMarker directory.

1. Click the **Produce Request String** button.
2. Copy the string in the text field and paste it into an email addressed to info@softgenetics.com for further order information. Another encrypted string of characters will be emailed to you.
3. Copy and paste the string into the second text field.
4. Click **Update** and the number of licenses in NetDog will automatically be updated.
5. Re-install the NetDog Server following the *Installation* section above.

Questions

If you have any questions during installation, setup, or program operation, please contact us at (814) 237-9340 *OR* (888) 791-1270 *OR* email us at tech_support@softgenetics.com

Chapter 2 General Procedure

Chapter 2 General Procedure

Import Data Files
Raw Data Analysis
Process Data
Adjust Analysis Parameters

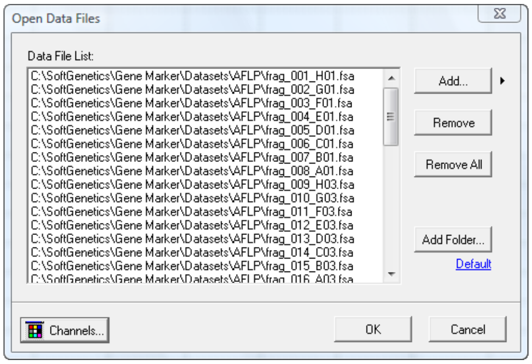
Import Data Files

After installing GeneMarker software you are ready to begin fragment analysis. First, raw data files must be uploaded to the program. Below is the list of file types supported by GeneMarker.

ABI - .fsa, .abi, .ab1, .hid
MegaBACE - .rsd
Beckman-Coulter - .esd
Spectrumedix - .smd
Generic - .scf, .sg1

Procedure

1. Launch **GeneMarker**
2. Click **Open Data**
3. The *Open Data Files* box will appear
4. Click **Add** button
5. The *Open* dialog will appear
6. Navigate to directory containing raw data files
7. Select all files by **CTRL+A** or use **CTRL** and/or **SHIFT** keys to select individual samples
8. Click **Open** button in the *Open* dialog
9. The files selected will appear in the *Data File List* field
10. Click **OK** button in the *Open Data Files* box and the samples will be uploaded to GeneMarker



```
C:\SoftGenetics\Gene Marker\Datasets\AFLP\frag_001_H01.fsa
C:\SoftGenetics\Gene Marker\Datasets\AFLP\frag_002_G01.fsa
C:\SoftGenetics\Gene Marker\Datasets\AFLP\frag_003_F01.fsa
C:\SoftGenetics\Gene Marker\Datasets\AFLP\frag_004_E01.fsa
C:\SoftGenetics\Gene Marker\Datasets\AFLP\frag_005_D01.fsa
C:\SoftGenetics\Gene Marker\Datasets\AFLP\frag_006_C01.fsa
C:\SoftGenetics\Gene Marker\Datasets\AFLP\frag_007_B01.fsa
C:\SoftGenetics\Gene Marker\Datasets\AFLP\frag_008_A01.fsa
C:\SoftGenetics\Gene Marker\Datasets\AFLP\frag_009_H03.fsa
C:\SoftGenetics\Gene Marker\Datasets\AFLP\frag_010_G03.fsa
C:\SoftGenetics\Gene Marker\Datasets\AFLP\frag_011_F03.fsa
C:\SoftGenetics\Gene Marker\Datasets\AFLP\frag_012_E03.fsa
C:\SoftGenetics\Gene Marker\Datasets\AFLP\frag_013_D03.fsa
C:\SoftGenetics\Gene Marker\Datasets\AFLP\frag_014_C03.fsa
C:\SoftGenetics\Gene Marker\Datasets\AFLP\frag_015_B03.fsa
C:\SoftGenetics\Gene Marker\Datasets\AFLP\frag_016_A03.fsa
```

Features

There are several features available in the *Open Data Files* box to make data upload easier.

Add…

Used to locate and select raw data files for upload. Click the arrow button next to the *Add* button to see the four most recently accessed directories.

Remove

Used to remove samples from the *Data File List*. Highlight the sample to remove by single left-clicking it in the *Data File List* then click **Remove**.

Remove All

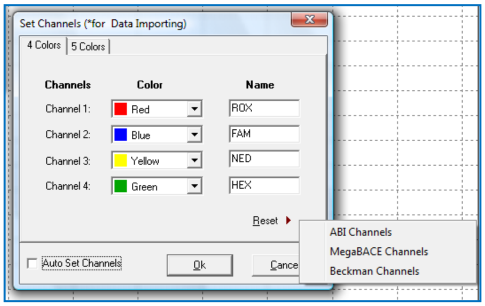
Removes all sample files from the *Data File List* field.

Add Folder…

Click **Add Folder** to upload raw data files from a specific folder in the file directory tree. Click the *Default* hyperlink to choose a folder to which GeneMarker will always open when the **Add** or **Add Folder** buttons are clicked.

Channels

Opens the *Set Channels* dialog with 4 and 5-color tab options and allows the user to choose from ABI, MegaBACE, and Beckman-Coulter standard dye color orders. The user can also manually enter dye color and name. The default channel color setup is ABI. Set the dye color channels before clicking **OK** in the *Open Data Files* dialog box.



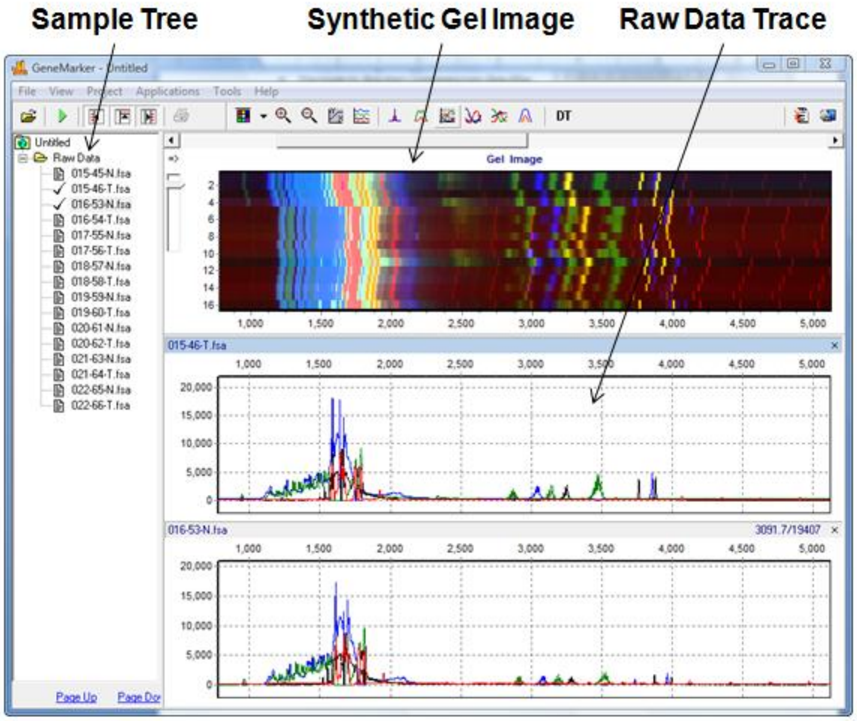
Channels	Color	Name
Channel 1:	🟥 Red	ROX
Channel 2:	🟦 Blue	FAM
Channel 3:	🟨 Yellow	NED
Channel 4:	🟩 Green	HEX

☐ Auto Set Channels

Raw Data Analysis

Once the raw data files are uploaded, the *Raw Data Main Analysis* window appears. Double-click the samples in the *Sample Tree* to open the individual *Raw Data Traces*. The *Synthetic Gel Image* displays the unprocessed data in a traditional gel format with larger fragments located on the right. The *Electropherograms* display fluorescent signal intensities as a single line trace for each dye color. The signal intensities, recorded in Relative Fluorescent Units (RFUs), are plotted along a frame scale in the *Raw Data Analysis* window with fragment mobility from right to left. The smallest size fragments are on the far left of the trace.

Raw Data Analysis Window


Sample Tree
Synthetic Gel Image
Raw Data Trace
GeneMarker - Untitled
File View Project Applications Tools Help
DT
Untitled
Raw Data
015-45-N.fsa
015-46-T.fsa
016-53-N.fsa
016-54-T.fsa
017-55-N.fsa
017-56-T.fsa
018-57-N.fsa
018-58-T.fsa
019-59-N.fsa
019-60-T.fsa
020-61-N.fsa
020-62-T.fsa
021-63-N.fsa
021-64-T.fsa
022-65-N.fsa
022-66-T.fsa
Page Up
Page Down
Gel Image
015-46-T.fsa
016-53-N.fsa
3091.7/19407
1,000 1,500 2,000 2,500 3,000 3,500 4,000 4,500 5,000
20,000 15,000 10,000 5,000 0

Main Toolbar Icons

Spike Removal: Removes peaks from voltage spikes caused by micro-air bubbles or debris in the laser path. This option is selected by default in the Run Wizard.

Saturation Correction: A synthetic peak is created based on peak shape before and after saturation. The results of these will be less accurate than that of non-saturated peaks. This option is selected by default in the Run Wizard.

Smooth: This function smoothes the baseline by eliminating smaller noise peaks. This option is selected by default in the Run Wizard.

Baseline Subtraction: Selecting this option will remove the baseline completely so that the Y-axis will be raised above the noise level. This option is selected by default in the Run Wizard.

Auto Pull-up Removal: Automatically removes peaks caused by wavelength bleed-through to other wavelengths. This option is selected by default in the Run Wizard.

Manual Pull-up Correction: This allows the user to manually adjust larger pull-up peaks in case the *Auto Pull-up Removal* function has not corrected the problem. It is recommend to de-select *Pull-up Correction* in the Run Wizard when using this function.

2nd Derivative Trace: This feature reduces high background noise and sharpens peaks. Baseline fluctuation caused from dye blobs or the DNA template in PCR can also be reduced with this function. It is recommended to de-select *Spike Removal* in the Run Wizard when this function has been activated.

What to Expect

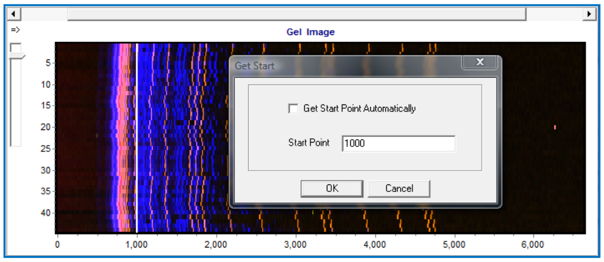
The raw data correction icons can be selected individually in the *Raw Data Analysis* window. The images below demonstrate how the data will look before (left image) and after (right image) the parameter is applied.

Range

AutoRange - Analyzes from 0 to end of trace for size call

Manual Range – user-defined range

Right-click in gel image and select **Get Start Point**



☐ Get Start Point Automatically

Start Point 1000

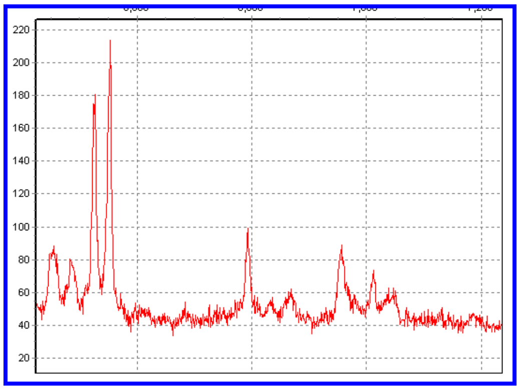
Smooth

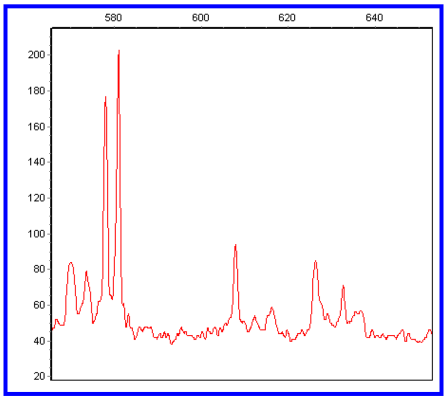
Fourier frequency transformation (FFT) to determine frequency domain

Use only top 40% of lowest frequencies

Smoothing broadens peaks and therefore you can lose resolution

Enhanced Smooth - Same as Smooth but use only top 20% of lowest frequencies

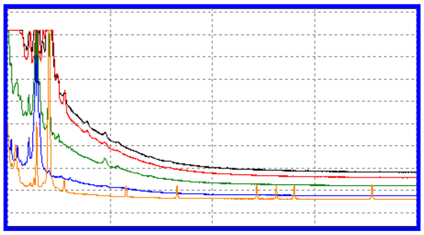


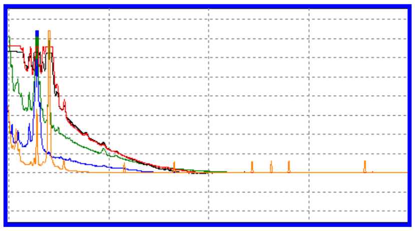


Baseline Subtraction

Use 20% of lowest intensities (to the right of the beginning of the range)

Looks at trace in 500-600 frame sections





Pullup Correction

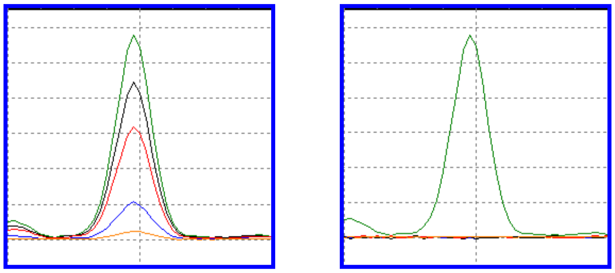
Ax=B

A being the major coefficient

Input matrix or use single dye adjustment up to 0.20 for small corrections

When **Manual Pullup** correction is chosen, a .txt or .mtx matrix file can be uploaded and used to deconvolute dye colors.

NOTE: De-select automatic *Pullup Correction* in the Run Wizard *Data Process* box if a manual matrix correction has been applied.



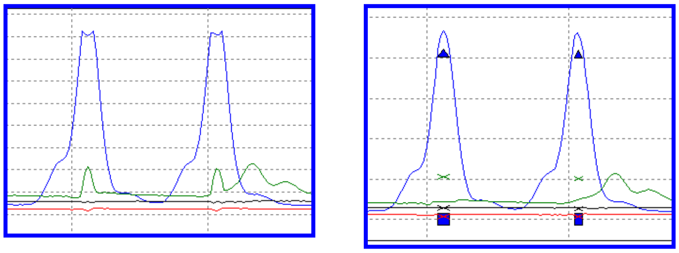
Saturated Peak Correction

ABI instrument saturated peaks are typically >8000 RFU

The top of a saturated peak looks split

A small pullup peak may be present under the saturated peak

GeneMarker takes the small pullup peak and adds it to the split in the saturated peak



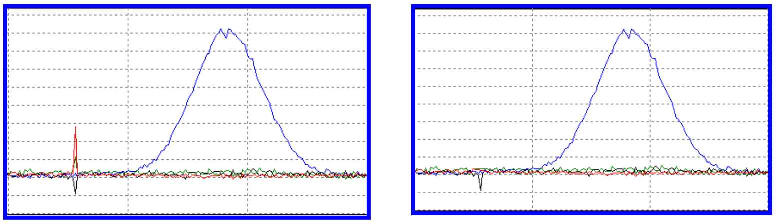
Spike Removal

Caused by overheating of camera chip, voltage spike, etc

Spikes usually only 1-2 frames wide; peaks usually 5-10 frames wide

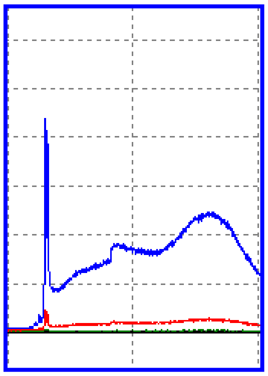
Create a first derivative trace of the raw data

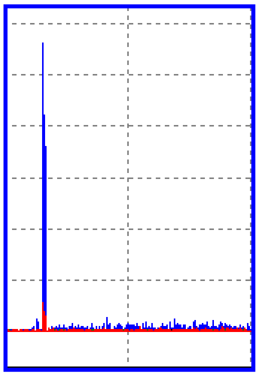
Spikes are the 1st DT outliers (3-5 sigma)



Second Derivative Trace

(A1-A2)-(A2-A3) = A1+A3-2(A2)
Use when you have a fat base to your peaks (ex. Dye blob under peak, etc)
NOTE: Do not use 2nd DT with *Spike Removal* because real peaks look like spikes.





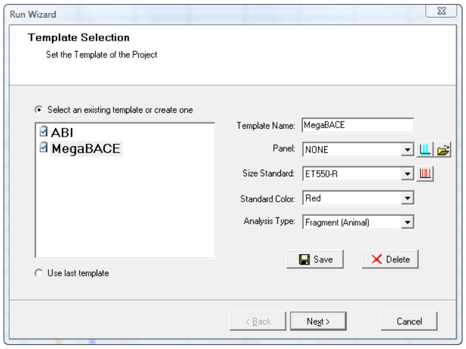
Process Data

After the raw data files have been uploaded to *GeneMarker*, they are ready to be processed. The processing step includes application of a sizing standard, filtering of noisy peaks, and comparison to a known allelic Panel if desired. *GeneMarker* combines all these steps in one simple tool called the **Run Wizard**. To access the Run Wizard simply click the **Run Project** icon in the main toolbar. The example below is for a basic fragment analysis. For advanced applications, **see Chapter 7 Special Applications.**

Run Wizard Template Selection

Procedure

1. Click the **Run Project** icon in the toolbar.
2. The Run Wizard *Template Selection* dialog box will appear.
3. Select a template (a previously saved set of size standard, standard color, and analysis type named for future use), *OR* select a new combination of size standard, standard color, and analysis type.
4. Click **Next** when finished.



● Select an existing template or create one

☑ ABI
☑ MegaBACE

Template Name: MegaBACE
Panel: NONE
Size Standard: ET550-R
Standard Color: Red
Analysis Type: Fragment (Animal)

○ Use last template

Icons and Functions

Template Name

Select from existing pre-made templates or create your own by entering a *Template Name* and clicking the **Save** button. To save the Run Parameters with the template use the 'Back' arrow after setting the parameters in the second and third screens of the Run Wizard. Then select 'Save' on the Tepmalte Selection screen.

To create a new template, click *Select an existing template or create one*. A template can also be selected from the list of available templates in the left section of the window and then saved for future use by clicking the **Save** button.

If you do not want to use a template, select the appropriate size standard, standard color, and type of analysis; *Use last template* will automatically be selected.

Panel

GeneMarker comes preloaded with many common kit Panels including Promega's MSI kit and MRC Holland's MLPA kits. Additional Panels can be imported by selecting the *Open Files* icon next to the *Panel* field. A custom Panel can be created in the *Panel Editor* tool. **See Chapter 5 Panel Editor.**
NOTE: It is recommended to size the data prior to selecting a Panel for comparison. Select **NONE** in the *Panel* field for the first Run Wizard data process action.

Panel Editor: A Panel can be selected from any available from the drop-down menu, or can be viewed and selected by clicking the *Panel Editor* icon.

Import a Panel: If a Panel cannot be found in the *Panel Editor* tool, it can be imported by clicking on the *Import a Panel* icon.

Size Standard

GeneMarker comes preloaded with many common size standards including GeneScan 500 and LIZ600. A custom Size Standard can be created by selecting the *Size Template Editor* icon next to the *Size Standard* field. **See Chapter 4 Fragment Sizing Standards.**

Size Template Editor: This allows the user to check sample files against a selected size standard, modify and save the size standard for future use, or create a new size standard.

Standard Color

Select the dye color which contains the internal lane standard.

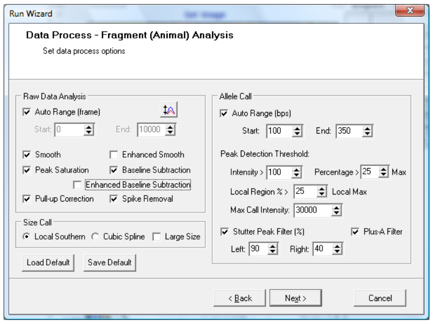
Analysis Type

Selecting an analysis type will set the options in the two subsequent boxes of Run Wizard *(Data Process* and *Additional Settings)* to the optimal settings for the analysis type selected. **See Chapter 7 Special Applications.**

Run Wizard Data Process

Procedure

5. The *Data Process* window of Run Wizard will activate. This allows the user to select the peak filtering parameters. The settings will vary depending on the *Analysis Type* selected.
6. Select the appropriate analysis settings in the *Data Process* window and click **Next** to continue.



Data Process - Fragment (Animal) Analysis

Set data process options

Raw Data Analysis
☑ Auto Range (frame)
Start: 0   End: 10000
☑ Smooth   ☐ Enhanced Smooth
☑ Peak Saturation   ☑ Baseline Subtraction
☐ Enhanced Baseline Subtraction
☑ Pull-up Correction   ☑ Spike Removal

Size Call
● Local Southern   ○ Cubic Spline   ☐ Large Size

Allele Call
☑ Auto Range (bps)
Start: 100   End: 350
Peak Detection Threshold:
Intensity > 100   Percentage > 25 Max
Local Region % > 25 Local Max
Max Call Intensity: 30000
☑ Stutter Peak Filter (%)   ☑ Plus-A Filter
Left: 90   Right: 40

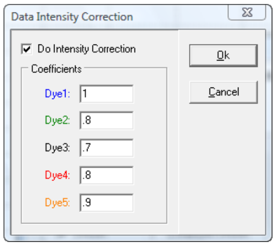
Icons and Functions

Raw Data Analysis

Auto Range (frame)

The range in camera frames will automatically find the processable data range. If *Auto Range* is not selected, manually enter the start and end frame numbers of the data set for analysis.

Intensity Coefficients: Allows for manual correction of excessive bleed-through peaks; best used for experiments with one-color analysis.



☑ Do Intensity Correction

Coefficients
Dye1: 1
Dye2: .8
Dye3: .7
Dye4: .8
Dye5: .9

Smooth

Smoothes the baseline by eliminating smaller noise peaks.

Enhanced Smooth

This feature is used only in cases where the data is extremely difficult to analyze and cannot be corrected with the *Smooth* function.

Peak Saturation

The software will analyze saturated data points by creating a synthetic estimate of the peak shape based on the curves prior to saturation. The results will be less accurate than that of non-saturated peaks.

Pull-up Correction

This function removes peaks caused by wavelength bleed-through to other wavelengths. The function should be disabled if a *Manual Pull-up Correction* was used in the *Raw Data Analysis* window.

Baseline Subtraction

This function removes the baseline completely so that the Y-axis will be raised above the noise level.

Enhanced Baseline Subtraction

This function should be applied for samples that have a high baseline, especially in the 40-80 bp mini-STR range.

Spike Removal
Removes peaks from voltage spikes caused by micro-air bubbles or debris in the laser path. Spikes are typically less than a base-pair wide. Do not select *Spike Removal* when 2^{nd} *Derivative Trace* has been applied.

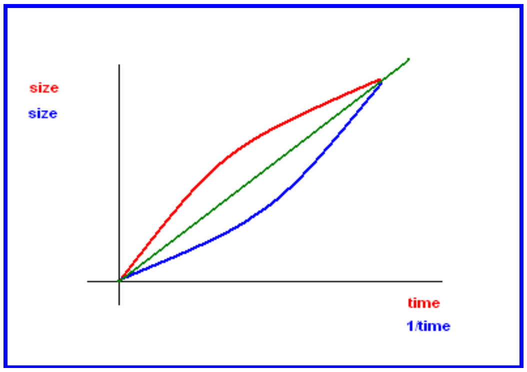
Size Call

GeneMarker offers three sizing methods:

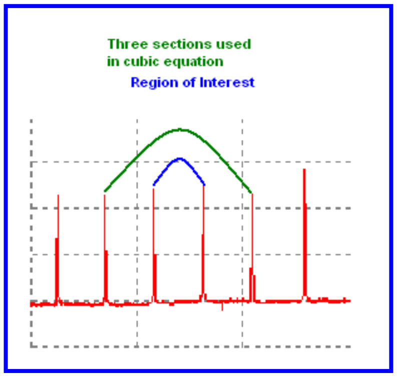
Local Southern
Used in most genotyping software applications and is recommended for most analyses. This method is based on the idea that smaller size fragments run faster. Plot a size v. time graph and overlay a size v. 1/time graph to determine linear trace. (Southern, E.M. "Measurement of DNA Length by Gel Electrophoresis." 1979. *Analytical Biochemistry*. **100**, 319-323).

Cubic Spline Method
Cubic Spline is offered as an alternative method that may be more appropriate for some data. This method uses a cubic equation to connect known points on the size v. time graph. An example of a cubic equation: ax^3+bx^2+cx+d. (*The Astrophysical Journal*. December 1, 1994. **436**, pages 787-794.)

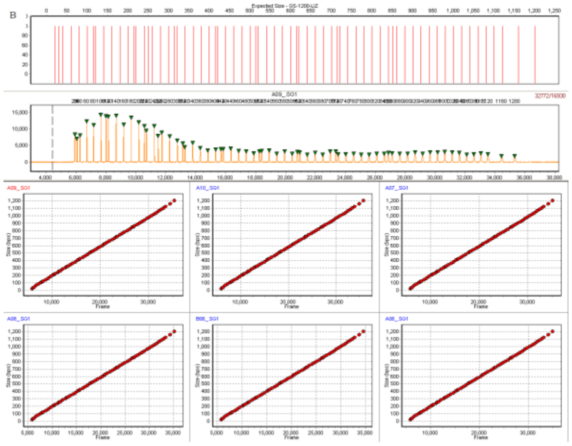
Local Southern Method



Cubic Spline Method



Large Size Algorithm GeneMarker's new algorithm provides accurate, linear sizing of the data using a DNA derivative migration time correction to large DNA fragments. Selecting Large Size algorithm enables accurate sizing from small to large (30-1400) base pair fragments. The peak area in base space accurately determines the copy numbers because the peak area normalization is much less variable than that of the height normalization. The peak width is less than 1 bps for all fragments less than 1k.



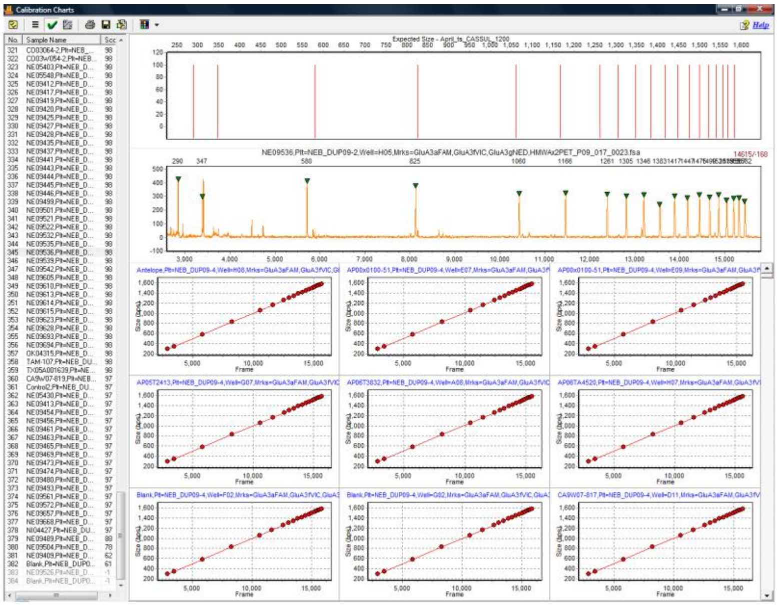
Custom Large Size Calling

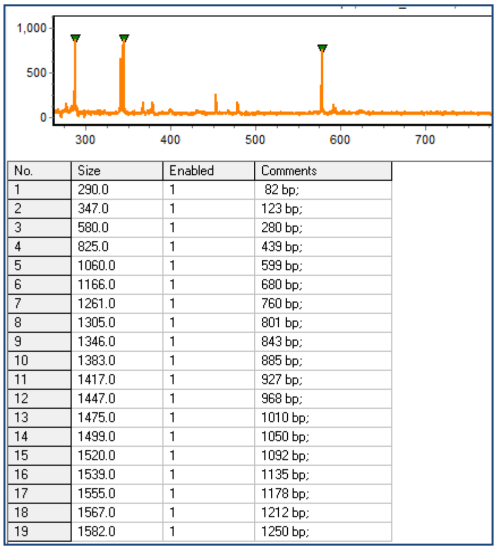
Some large custom size standards have unique size fragments that are not evenly spaced; requiring the ability to change some of the parameters in the large size calling algorithm to provide a best fit to the fragments. The large size calling algorithm (above) will use analyst supplied parameters to provide accurate linear sizing of the custom large size standard. Use the spread sheet in the C:ProgramFiles:SoftGeneticsGeneMarkerSize Standard folder to assist in determining the appropriate parameters for custom large size standards.

Flanking Peak Size Calling

With some non-linear data it is not possible to make a best fit curve using the algorithms above. The flanking peak method uses the fragments on either side of the data peak to interpolate the size. This allows size calling of non-linear data. To create the size standard for this method:

1. Import raw data files and Select None for Panel and Size Standard in the Run Wizard
2. Select Tools → Size Template Editor
3. Select File → New Size Standard
4. Enter the 'Size' information from the run without a size standard in the Size column of the Size standard
5. Enter the Expected size bp; in the comments column as seen in the figure below
6. Save the size standard file
7. Select Local Southern method to activate this size calling method in the Run Wizard

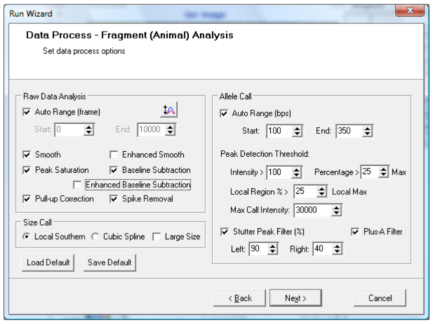




No.	Size	Enabled	Comments
1	290.0	1	82 bp;
2	347.0	1	123 bp;
3	580.0	1	280 bp;
4	825.0	1	439 bp;
5	1060.0	1	599 bp;
6	1166.0	1	680 bp;
7	1261.0	1	760 bp;
8	1305.0	1	801 bp;
9	1346.0	1	843 bp;
10	1383.0	1	885 bp;
11	1417.0	1	927 bp;
12	1447.0	1	968 bp;
13	1475.0	1	1010 bp;
14	1499.0	1	1050 bp;
15	1520.0	1	1092 bp;
16	1539.0	1	1135 bp;
17	1555.0	1	1178 bp;
18	1567.0	1	1212 bp;
19	1582.0	1	1250 bp;

Allele Call

The *Allele Call* section allows the user to set allele calling range, detection thresholds and filters.



Data Process - Fragment (Animal) Analysis

Set data process options

Raw Data Analysis
☑ Auto Range (frame) Start: 0 End: 10000
☑ Smooth ☐ Enhanced Smooth
☑ Peak Saturation ☑ Baseline Subtraction
☐ Enhanced Baseline Subtraction
☑ Pull-up Correction ☑ Spike Removal

Size Call
● Local Southern ○ Cubic Spline ☐ Large Size

Allele Call
☑ Auto Range (bps) Start: 100 End: 350
Peak Detection Threshold:
Intensity > 100 Percentage > 25 Max
Local Region % > 25 Local Max
Max Call Intensity: 30000
☑ Stutter Peak Filter (%) ☑ Plus-A Filter
Left: 90 Right: 40

Auto Range

The software will automatically display the processable data range for each lane.

Manual selection of Range

To select a specific analysis region, de-select *Auto Range* and input the desired base pair range. Additionally, if automatic size call fails due to high saturation, de-select *Auto Range* and manually input the required data range.

Peak Detection Threshold
The recommended initial settings are different for each analysis type. Please see the appropriate chapters for the required analysis type.

Intensity
Minimum RFU threshold of peak height used for peak detection. Peaks below this value will not be called.

Percentage
Relative minimum intensity of allele peaks to the 5th highest peak in the dye color used for peak detection.

Local Region Percent
Uses the percentage of the highest peak for that marker to define the minimum peak detection threshold. For example, if the threshold is set to 33%, the height of all allele peaks must reach at least 33% of the height of the highest peak in that particular locus to be called.

Max Call Intensity
Maximum RFU threshold of peak height used for peak detection. Peaks above this value will not be called.

Stutter Peak Filter
Forward and reverse stutter peaks commonly caused by PCR/chemical reactions can be removed using the *Stutter Peak Filter*. The settings are in percentage of the primary peak. The default settings will remove stutter peaks within 2.5 base pairs of the primary peak.

Plus A Filter
When selected, the second peak of a split peak (one basepair spacing between two peaks) will not be called. Deselect to call both peaks separately.

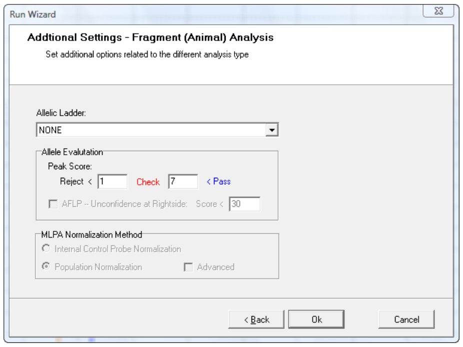
Load Default
Recalls any settings previously saved by the user. If there are no user-saved settings, the program loads the default settings for that particular analysis type.

Save Default
Saves any settings defined by the user that is different from the default. These settings can be recalled for consistency of analysis on similar data sets.

Run Wizard Additional Settings

Procedure

7. The Run Wizard is almost complete. Enter or change the appropriate information for your analysis type.
8. Once the settings have been adjusted to your analysis type, click **OK** and a *Data Processing* window will appear while GeneMarker sizes the data based on the parameters set in the Run Wizard. Click **OK** when the analysis has finished.


Run Wizard

Addtional Settings - Fragment (Animal) Analysis

Set additional options related to the different analysis type

Allelic Ladder: NONE

Allele Evalutation

Peak Score:

Reject < 1 Check 7 < Pass

☐ AFLP -- Unconfidence at Rightside: Score < 30

MLPA Normalization Method

○ Internal Control Probe Normalization

● Population Normalization ☐ Advanced

Functions

Allelic Ladder

Permits the selection of a sample containing an allelic ladder. This option is only available when *Fragment (Animal)* or *HID* analysis types are selected.

Allele Evaluation

Peak Score
User-definable confidence level of the allele call. Peak score is an algorithm that takes into account signal-to-noise ratio and peak morphology. Rejected samples appear in red, samples that need to be checked appear in yellow, and samples that have passed appear in green.

AFLP Unconfidence at Rightside Score

Available only when AFLP Analysis method is chosen. When selected the user can enter the minimum score at which a peak to the right of the main peak will be called. **See Chapter 7 Special Applications - AFLP**.

MLPA Normalization Method
Available only when MLPA Analysis method is chosen. Two normalization methods are available to correct for variations of PCR efficiencies from small to large DNA fragments or from sample to sample. **See Chapter 7 Special Applications MLPA.**

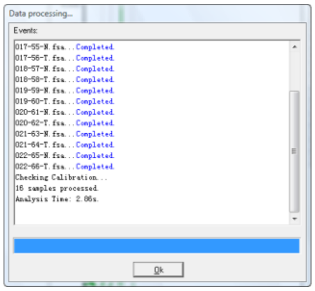
Internal Control Probe Normalization
The traditional method of normalization using the control probes.

Population Normalization
Unique to *GeneMarker*, this method normalizes peak intensities based on the statistically most probable median intensities.

Advanced
Available when *Population Normalization* is selected. *Advanced Population Normalization* is meant to be used when more than half of the probes are deletions or duplications.

Adjust Analysis Parameters

After the clicking **OK** in the Run Wizard *Additional Settings* box, the *Data Processing* box appears. The raw data is being processed and sized, then the filtering parameters are applied, and finally a Panel is applied (if selected). Click **OK** in the *Data Processing* box when analysis is complete.



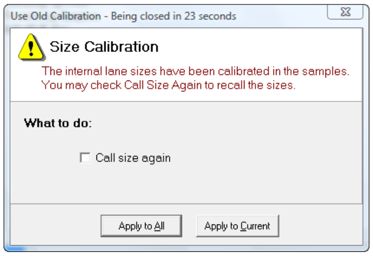
```
017-55-N.fsa...Completed.
017-56-T.fsa...Completed.
018-57-N.fsa...Completed.
018-58-T.fsa...Completed.
019-59-N.fsa...Completed.
019-60-T.fsa...Completed.
020-61-N.fsa...Completed.
020-62-T.fsa...Completed.
021-63-N.fsa...Completed.
021-64-T.fsa...Completed.
022-65-N.fsa...Completed.
022-66-T.fsa...Completed.
Checking Calibration...
16 samples processed.
Analysis Time: 2.86s.
```

Review the results in the *Main Analysis* window. **See Chapter 3 Main Analysis Overview.** If you notice many false positive peak calls, you may need to adjust the analysis parameters. There are three options for adjusting the analysis parameters as discussed below.

NOTE: Manual edits will be lost when data is re-analyzed.

Re-analyze with Run Wizard

To re-analyze with the Run Wizard tool, simply click the **Run Project** icon in the main toolbar. The Run Wizard will launch and the most recently selected parameters will be displayed. Adjust parameters as necessary and click **OK** in the Run Wizard *Additional Settings* box. The *Use Old Calibration* box will appear with the option to **Call Size Again**. Only select *Call Size Again* if the Run Wizard *Template Selection Size Standard* selection was changed or any of the Run Wizard *Data Process Raw Data Analysis* parameters were changed. Click the **Apply to All** button. The *Data Processing* box will appear again and the data will be re-analyzed with the new parameters.



The internal lane sizes have been calibrated in the samples.
You may check Call Size Again to recall the sizes.

☐ Call size again

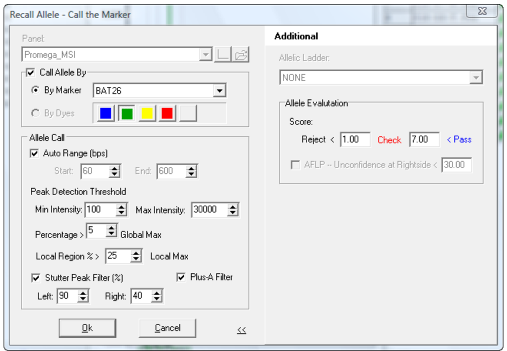
Re-analyze with Auto Run

To re-analyze with Auto Run, first select *Project → Options*. The *Project Options Settings* box will appear. This box offers all the same parameters settings as are available in the Run Wizard. Use the tabs to view the *Template Selection, Data Process*, and *Additional Settings* boxes. Click **OK** when finished. Next, select *Project → Auto Run*. The data will be re-analyzed with the new parameters.
NOTE: The *Additional Settings Allele Evaluation Peak Score* parameters can be changed in the *Project Options Settings* box and will be applied to the data without having to re-analyze the data with Run Wizard or Auto Run.

Re-analyze Individual Samples

To re-analyze an individual sample, dye color, or marker, click the **Call Allele** icon in the main toolbar. The arrow next to the icon opens the drop-down menu with additional options. Click an option from the drop-down and the *Recall Allele* box will appear. Adjust parameters as necessary and click **OK**. The new parameters will be applied.

All Samples
Applies the new parameter settings to all samples in the dataset – similar to Run Wizard and Auto Run.



Panel: Promega_MSI
☑ Call Allele By
● By Marker BAT26
○ By Dyes 🟦 🟩 🟨 🟥
☑ Auto Range (bps)
Start: 60 End: 600
Min Intensity: 100 Max Intensity: 30000
Percentage > 5 Global Max
Local Region % > 25 Local Max
☑ Stutter Peak Filter (%) ☑ Plus-A Filter
Left: 90 Right: 40
Allelic Ladder: NONE
Reject < 1.00 Check 7.00 < Pass
☐ AFLP -- Unconfidence at Rightside < 30.00

Open Samples
Applies the new parameter settings only to samples that are checked in the *Sample File Tree*.

Current Sample
Applies the new parameter settings only to the sample highlighted in the *Sample File Tree*.

Call the Dye
Applies the new parameter settings to the dye selected in the *Recall Allele* → *Call Allele by Dye* field.

Call the Marker
Applies the new parameter settings to the marker selected in the *Recall Allele* → *Call Allele by Marker* field.

Chapter 3 Main Analysis Overview

Chapter 3 Main Analysis Overview

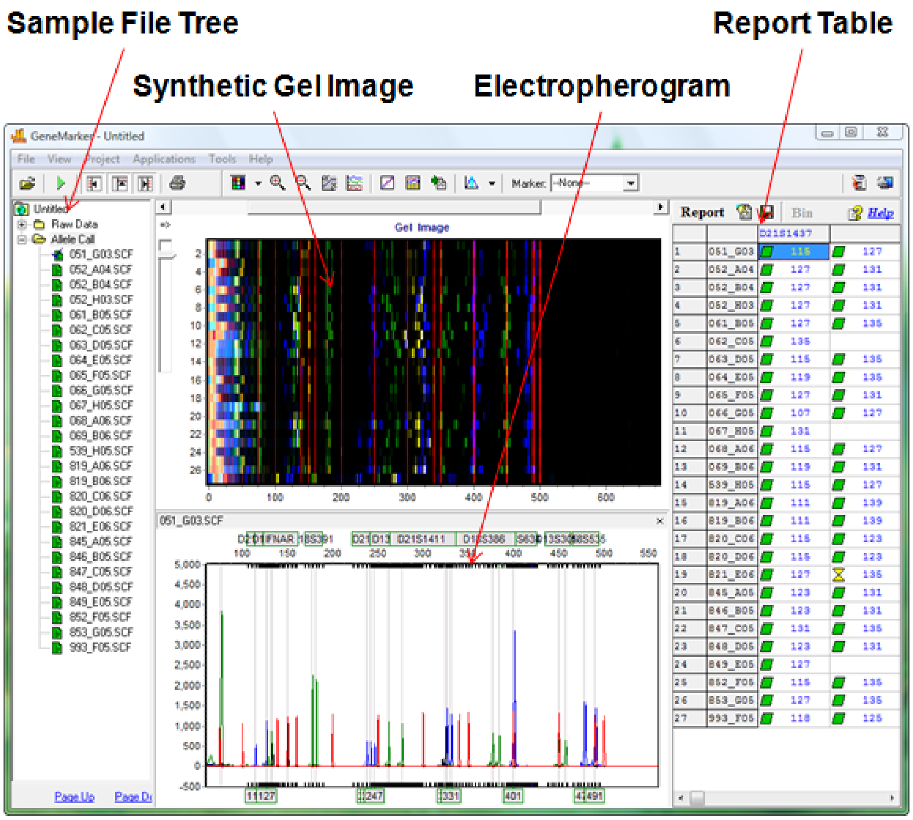
Main Analysis Window
Menu Options
Main Toolbar Icons

Main Analysis Window

The main window of GeneMarker has an easy to use layout. The sample files are displayed on the left, the *Synthetic Gel Image* is displayed at the top, *Electropherograms* appear below the gel image, and the *Report Table* is on the right side of the window.

To resize the frames in the *Main Analysis* window, simply place the cursor over the partitions that separate the *Synthetic Gel Image/Electropherogram/Sample File Tree/Report Table*. The cursor will change to a two-headed arrow bisected by two vertical lines. Hold down the left mouse button and drag the gray vertical line in the direction you wish. To open and close the frames, use the **Show/Hide** icons in the main toolbar.

Main Analysis Window

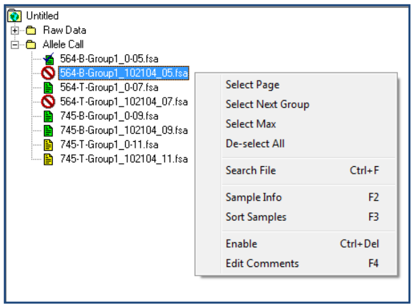

Sample File Tree
Report Table
Synthetic Gel Image
Electropherogram
GeneMarker - Untitled
File View Project Applications Tools Help
Marker: --None--
Untitled
Raw Data
Allele Call
051_G03.SCF
052_A04.SCF
052_B04.SCF
052_H03.SCF
061_B05.SCF
062_C05.SCF
063_D05.SCF
064_E05.SCF
065_F05.SCF
066_G05.SCF
067_H05.SCF
068_A06.SCF
069_B06.SCF
539_H05.SCF
819_A06.SCF
819_B06.SCF
820_C06.SCF
820_D06.SCF
821_E06.SCF
845_A05.SCF
846_B05.SCF
847_C05.SCF
848_D05.SCF
849_E05.SCF
852_F05.SCF
853_G05.SCF
993_F05.SCF
Page Up
Page Dn
Gel Image
051_G03.SCF
D2D1FNAR
D8S391
D21D13
D21S1411
D18S386
Report
Bin
Help

		D21S1437	
1	051_G03	🟩 115	🟩 127
2	052_A04	🟩 127	🟩 131
3	052_B04	🟩 127	🟩 131
4	052_H03	🟩 127	🟩 131
5	061_B05	🟩 127	🟩 135
6	062_C05	🟩 135	
7	063_D05	🟩 115	🟩 135
8	064_E05	🟩 119	🟩 135
9	065_F05	🟩 127	🟩 131
10	066_G05	🟩 107	🟩 127
11	067_H05	🟩 131	
12	068_A06	🟩 115	🟩 127
13	069_B06	🟩 119	🟩 131
14	539_H05	🟩 115	🟩 127
15	819_A06	🟩 111	🟩 139
16	819_B06	🟩 111	🟩 139
17	820_C06	🟩 115	🟩 123
18	820_D06	🟩 115	🟩 123
19	821_E06	🟩 127	⧗ 135
20	845_A05	🟩 123	🟩 131
21	846_B05	🟩 123	🟩 131
22	847_C05	🟩 131	🟩 135
23	848_D05	🟩 123	🟩 131
24	849_E05	🟩 127	
25	852_F05	🟩 115	🟩 135
26	853_G05	🟩 127	🟩 135
27	993_F05	🟩 118	🟩 125

Sample File Tree

The *Sample File Tree* of the main analysis window contains two folders. The first is the **Raw Data** folder which, when expanded, displays a list of all the dataset samples. When a sample is double-clicked its preprocessed electropherogram trace will appear in the *Raw Data Analysis* window. **See Chapter 2 General Procedure.**

The second folder, **Allele Call**, also contains a list of all the samples, but when the filename is double-clicked the sample's electropherogram trace appears in the *Main Analysis* window with all sizing information and allele call filtering applied. The Allele Call folder also flags each sample with a green sheet, yellow sheet or red strike-through indicating size calling success. **See Chapter 4 Fragment Sizing Standards.**


Untitled
Raw Data
Allele Call
564-B-Group1_0-05.fsa
564-B-Group1_102104_05.fsa
564-T-Group1_0-07.fsa
564-T-Group1_102104_07.fsa
745-B-Group1_0-09.fsa
745-B-Group1_102104_09.fsa
745-T-Group1_0-11.fsa
745-T-Group1_102104_11.fsa
Select Page
Select Next Group
Select Max
De-select All
Search File Ctrl+F
Sample Info F2
Sort Samples F3
Enable Ctrl+Del
Edit Comments F4

Right-click the sample filename in the *Raw Data* or *Allele Call* folder to see additional options.

Sorting Options

Select Page

Opens electropherogram traces for the number of samples specified in the *View → Preference → Display Settings → Max Chart # In Page* field. **Hot Key = Page Up/Page Down**

Select Next Group

In descending order, selects the same number of samples previously selected by *Select Page*, grouping options (see *Sample Grouping* section below) or double-click option.

Select Max

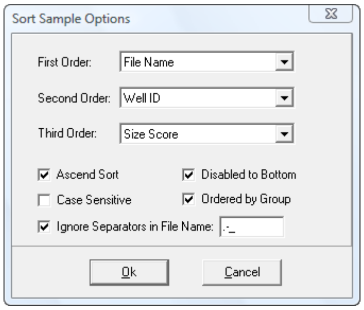
Opens electropherogram traces for the number of samples specified in the *View → Preference → Display Settings → Max # Open Charts.*

Deselect All

Unselects all selected samples in the *Sample File Tree* list and closes the electropherogram traces.

Sort Samples

Opens the *Sort Sample Options* box. Select First, Second, and Third Order sorting from the drop-down menu options Sample Type, File Name, Lane Number, Well ID, and Size Score. **Hot Key = F3**



First Order: File Name

Second Order: Well ID

Third Order: Size Score

☑ Ascend Sort
☑ Disabled to Bottom
☐ Case Sensitive
☑ Ordered by Group
☑ Ignore Separators in File Name: .-_

Search Options

Search File

Opens the *File Search* box. Enter any part of a filename to search for the sample in the list. Click the **Search** button. Left-click and use **CTRL** or **SHIFT** key to highlight samples then click the **Open Selected** button. The electropherograms of the selected samples will open in the *Main Analysis* window. **Hot Key = CTRL+F**

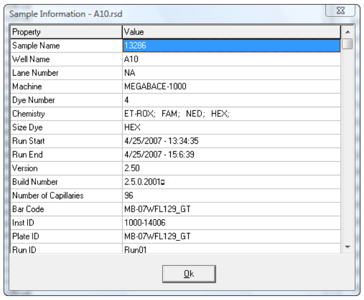
Sample Information

Sample Info

Opens the *Sample Information* box. A list of *Properties* appears and includes information like; Sample Name, Well ID, Lane Number, Instrument Name, and Chemistry. The list of *Properties* varies depending on the file type. **Hot Key = F2**

Edit Comments

Opens the *Edit Comments* box. Enter information in the *Comments* field. The last ten comments will be stored and can be subsequently selected for future samples. The *Sample Comments* will appear on the *Print Report*. **See Chapter 6 Reports and Printing.** **Hot Key = F4**

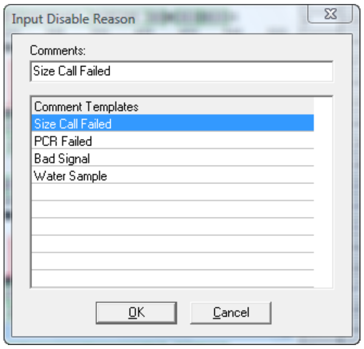


Property	Value
Sample Name	13286
Well Name	A10
Lane Number	NA
Machine	MEGABACE-1000
Dye Number	4
Chemistry	ET-ROX; FAM; NED; HEX;
Size Dye	HEX
Run Start	4/25/2007 - 13:34:35
Run End	4/25/2007 - 15:6:39
Version	2.50
Build Number	2.5.0.2001
Number of Capillaries	96
Bar Code	MB-07WFL129_GT
Inst ID	1000-14006
Plate ID	MB-07WFL129_GT
Run ID	Run01

Disable Samples

Disable Sample

Opens the *Input Disable Reason* box and marks the sample with a red strike-through. A disabled sample cannot be selected for display in the *Main Analysis* window and will not appear in the *Report Table* if *View → Preference → Options → Show Disabled Samples in Report* is deselected. Select a *Comment Template* or enter a new comment in the *Comments* field and click **OK** to disable the sample. **Hot Key = CTRL+DEL**



Comments: Size Call Failed

Comment Templates
- Size Call Failed
- PCR Failed
- Bad Signal
- Water Sample

Add Samples

From the main toolbar, select *Project → Add Samples to Project*. The *Open Data Files* box will appear. Click the **Add** button to select additional samples to add to the project and click **OK**. The added samples will be sized and the allele calls will be filtered according to the parameters set in the Run Wizard.

Sample Grouping

From the main toolbar, select *Project → Apply Sample Grouping*. The *File Name Group Editor* tool will appear (**See Chapter 8 Additional Tools**). Select *Group* and *Control* identifiers and click **Match**. Click **OK** to apply the matched groups. Group numbers will appear next to the filenames in the *Sample File Tree*. Use the **Select Next**

Group right-click menu option OR **CTRL+PageUp/Down** to open samples in a group. To disable the Sample Grouping feature, go to *View → Preference → Others* and uncheck *Enable Sample Grouping.*

Synthetic Gel Image and Electropherogram with Peak Table

The *Synthetic Gel Image* and *Electropherogram* displays are associated in the *Main Analysis* window. Both display the fragment information in a visual form. When GeneMarker is initially launched, all dye colors are displayed in the *Synthetic Gel Image* and *Electropherogram* at once. Single left-click the **Show Color** icon in the main toolbar to cycle through the dye colors or use the **Show Color** drop-down menu to disable individual colors or **Show/Hide All** colors.

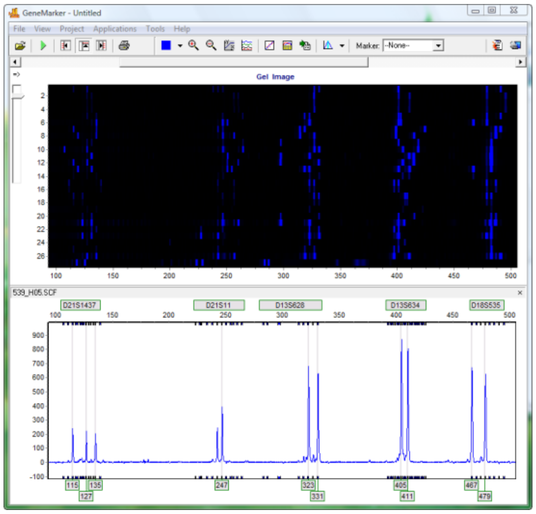
Navigation

Zoom In/Out

In the *Synthetic Gel Image* or the *Electropherogram*, hold down the left mouse button and drag a box from upper left to lower right around the area you would like to zoom in on. To zoom back out, hold down the left mouse button and drag a box in the opposite direction from lower right to upper left. The user may also use the Zoom icons in the main toolbar to zoom in and out. The main analysis window also allows the user to manually set the x and y axis with the **Set Axis** icon.

Horizontal Movement

The *Synthetic Gel Image* and the *Electropherogram* are synchronized to allow the user to view both images at once. To move the images in the horizontal direction, use the top slider bar (below the toolbar) to scroll the image in either direction, or hold down the **right mouse button** and drag the trace right or left.


GeneMarker - Untitled
File
View
Project
Applications
Tools
Help
Marker:
--None--
Gel Image
2
4
6
8
10
12
14
16
18
20
22
24
26
100
150
200
250
300
350
400
450
500
539_H05.SCF
D21S1437
D21S11
D13S628
D13S634
D18S535
100
150
200
250
300
350
400
450
500
900
800
700
600
500
400
300
200
100
0
-100
115
127
135
247
323
331
405
411
467
479

Marker/Locus Specific Viewing

To scroll through individual markers/loci, select a marker from the *Marker* drop-down list in the main toolbar. To view subsequent markers, use the **Up/Down Arrow** keys.

Synthetic Gel Image Features

The *Synthetic Gel Image* displays all samples in the dataset vertically. The direction of fragment mobility is horizontal with the small size fragments on the left and the larger fragments on the right so that the gel aligns with the electropherogram trace display. Move the mouse pointer over the *Synthetic Gel Image* to reveal the sample lane filename.

Image Utilities

Click the **Image Utilities** icon in the upper left corner of the *Synthetic Gel Image*. A fly-out menu appears with the following options:

Copy to Clipboard

Copies the *Synthetic Gel Image* to the Windows clipboard for pasting into other applications such as Microsoft PowerPoint.

Save Image

Allows the user to save the *Synthetic Gel Image* as a BMP image file.

Show in Window

Opens a separate window containing the *Synthetic Gel Image*. The separate window can be maximized for closer gel image inspection.

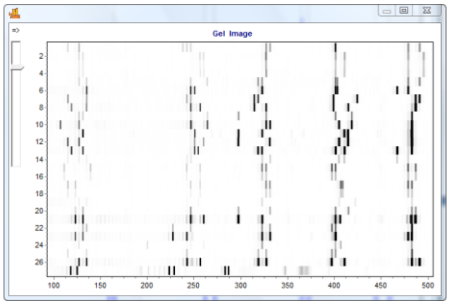
Image Display

Intensity

Move the Intensity slide bar, located in the upper left corner of the *Synthetic Gel Image*, up and down to adjust the intensity of the fragments displayed.

Grey-Scale

Go to *View* → *Preference* → *Display Settings* → *Gel Image*. Select **Gray for Single Dye** to change the single dye *Synthetic Gel Image* to black and white when only a single dye color is selected (when multiple dye colors are selected the fragments will appear in their respective colors). Click the **Background in White** option to reverse the black and white exposure for single dye color gel images.


Gel Image
2
4
6
8
10
12
14
16
18
20
22
24
26
100
150
200
250
300
350
400
450
500

Electropherogram and Peak Table Features

The *Electropherogram* displays fluorescent signal intensities from capillary electrophoresis instruments as a single line trace for each dye color. The signal intensities are recorded in Relative Fluorescent Units (RFUs) which are plotted along the y-axis. Along the x-axis are the basepair sizes of the fragments. The frame units plotted along the x-axis in the original *Raw Data Analysis* window are converted to basepair size units as defined by the Size Standard selected and the Internal Lane Standard (ILS) of the individual samples. Fragment mobility is from right to left with the smallest size fragments on the far left of the trace.

The *Peak Table* contains information about the called peaks currently displayed in the *Electropherogram*.

Electropherogram Trace Display

Range

The basepair size range (x-axis) is as set in the Run Wizard *Data Process Allele Call* options box. The RFU range (y-axis) is variable and will re-adjust according to the maximum peak height in the trace. To manually set x and y-axis ranges, use the **Set Axis** icon in the main toolbar.

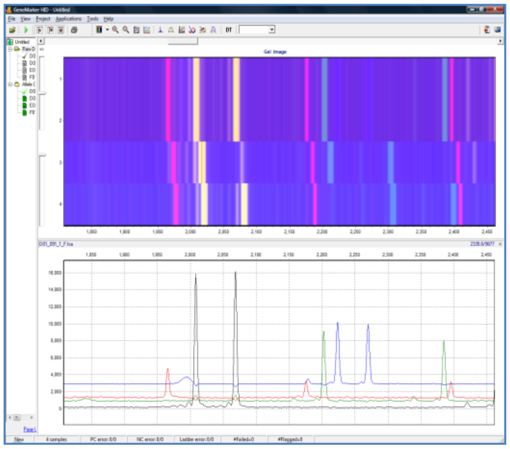
Cursor Locator

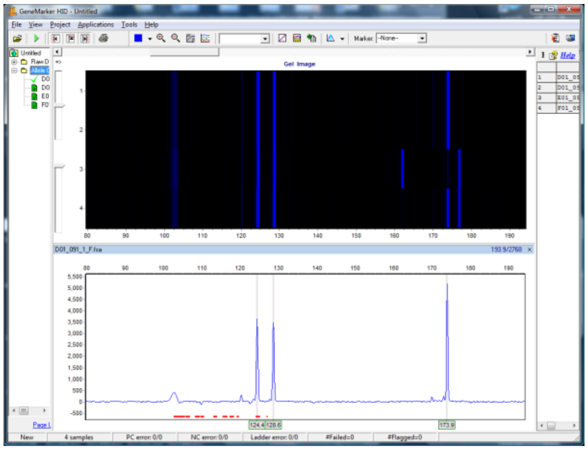
The x and y-axis position of the mouse pointer in the electropherogram is displayed in the upper right corner of the electropherogram.

Allele Call

If a Panel is applied to the data, then grey horizontal bar *Markers* will appear above the electropherogram indicating locus ranges. *Bin* ranges appear as dye-colored brackets above and below the sample trace. *Allele Labels* appear below the electropherogram and are associated with the center of each called peak which is also marked by a light grey vertical line in the electropherogram. If a Panel is not applied, then *Allele Labels* for called peaks will only indicate the basepair size of the peak.

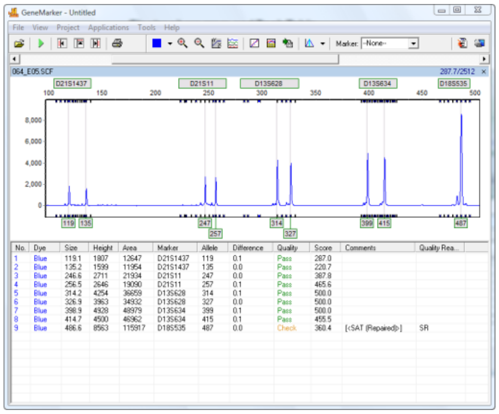
The red horizontal line (seen here in the figure on the right) is to alert analysts to trends in the data. These areas of the data have a more elevated baseline or noise-to-signal ratio (often associated with poorly resolved peaks) than the nearby regions of the trace which sometimes masks very minor peaks.


GeneMarker HID - Untitled
File View Project Applications Tools Help
Gel Image


GeneMarker HID - Untitled
File View Project Applications Tools Help
Marker: -None-
Gel Image
Help
D01_091_1_F.fsa
124.4
128.6
173.9
4 samples
PC error: 0/0
NC error: 0/0
Ladder error: 0/0
#Failed=0
#Flagged=0

Peak Table

The *Peak Table* can be displayed below the *Electropherogram* by clicking the **Show Chart/Table** icon in the main toolbar. Right-click in the *Peak Table* and select **Show Columns**. The *Show Columns* fly-out appears with column options.



No.	Dye	Size	Height	Area	Marker	Allele	Difference	Quality	Score	Comments	Quality Rea...
1	Blue	119.1	1807	12647	D21S1437	119	0.1	Pass	287.0		
2	Blue	135.2	1599	11954	D21S1437	135	0.0	Pass	220.7		
3	Blue	246.6	2711	21934	D21S11	247	0.0	Pass	387.8		
4	Blue	256.5	2646	19090	D21S11	257	0.1	Pass	465.6		
5	Blue	314.2	4254	36659	D13S628	314	0.1	Pass	500.0		
6	Blue	326.9	3963	34932	D13S628	327	0.0	Pass	500.0		
7	Blue	398.9	4928	48979	D13S634	399	0.1	Pass	500.0		
8	Blue	414.7	4500	46962	D13S634	415	0.1	Pass	455.5		
9	Blue	486.6	8563	115917	D18S535	487	0.0	Check	360.4	[<SAT (Repaired)>]	SR

Dye
Indicates the dye color of the peak.

Size
Indicates the basepair size of the peak (x-axis).

Height
Indicates the peak height in RFUs (y-axis).

Height Ratio
The value obtained when the peak's height is divided by the height of the highest peak in the dye color or Marker.

Area
Indicates the area under the curve of the peak. The area calculation begins and ends along the x-axis as indicated by the *Start* and *End* columns of the *Peak Table* respectively.

Area Ratio
The value obtained when the peak's area is divided by the area of the highest peak in the dye color or Marker.

Marker *(Panel Only)*
Indicates which *Marker* (Locus) the peak is contained in.

Allele *(Panel Only)*
Indicates which *Bin* the peak is contained in.

Difference *(Panel Only)*
Indicates the absolute value in basepairs of how far the peak center is from the *Bin* center.

Quality *(Panel Only)*
Assigns a *Pass/Check/Undetermined* quality ranking for each peak with regard to the peak *Score* as set in the Run Wizard *Additional Settings* box (**See Chapter 2 General Procedure**) and/or software editing of the original raw data, such as correction of saturated peaks (SAT Repaired).

Score
The peak quality score is calculated based on signal-to-noise ratio and peak shape or morphology. Lower scores indicate poorer quality peaks. Additionally, the *Score* value is a based on an exponential curve.

Start/End
Indicate the beginning and end of the *Area* calculation for the peak.

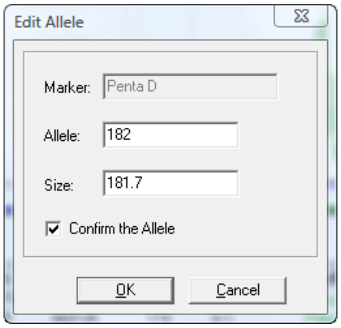
Comments
Software and user edited comments appear in the *Comments* column.

Quality Reasons
Indicates the reason why a peak received a Quality rank of *Check* or *Undetermined*. For explanation of the two and three letter codes, click the **Help** icon above the *Report Table*.

Save Peak Table
Click the **Save Peak Table** icon in the main toolbar to export the *Peak Table* information currently being displayed in Excel (.xls) or tab-delimited Text (.txt) format. All samples peak information for only the dye colors selected will be exported in the table. Additionally, the user can right-click in the *Peak Table* and select **Copy Table** (**Hot Key** = **CTRL+C**) to place the current table information onto the Windows clipboard. The information can then be pasted into most common spreadsheet or word processing programs including Microsoft Excel.

Editing Peaks

Double-click the vertical grey peak center bar to select a peak. Right-click anywhere in the *Electropherogram* or *Peak Table* to see additional menu options.



Marker: Penta D

Allele: 182

Size: 181.7

☑ Confirm the Allele

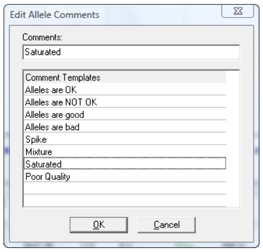
Insert Allele
Right-click at the place in the electropherogram where you would like to add an allele and select *Insert Allele*. The basepair size or bin name will be applied in the *Allele Label* and the peak specifications will be calculated and displayed in the *Peak Table*.

Delete/Undelete Allele
Right-click at the vertical grey bar indicating the center of the called peak or the peak cell in the *Peak Table* and select *Delete* (**Hot Key = DEL**). To call the allele again, right-click the peak and select *Undelete* (**Hot Key = SHIFT+DEL**).

Confirm/Unconfirm Allele
If a peak is given a low quality score, it will receive a *Check* (yellow) or *Undetermined* (red) Quality ranking. To give the peak a *Pass* (green) Quality ranking, right-click the peak center bar and select *Confirm* (**Hot Key = CTRL+M**). The peak will be marked *Pass* (green) and receive a “Confirmed” comment in the *Peak Table*. To un-confirm the allele, select *Unconfirm* from the right-click menu (**Hot Key = CTRL+ALT+M**).

Confirm/Unconfirm All
Confirm All and *Unconfirm All* options perform the same actions as the *Confirm/Unconfirm* allele except that the Quality ranking for all peaks in that dye color for that sample will be affected.

Edit Allele
Right-click an allele in the *Electropherogram* or *Peak Table* and select *Edit Allele*. The *Edit Allele* box appears. Add or change the values in the *Allele* and/or *Size* field. The *Allele* field will be blank if no Panel has been applied to the dataset. Check *Confirm the Allele* to automatically give the peak a Quality rank of *Pass* (green).



Comments: Saturated

Comment Templates
Alleles are OK
Alleles are NOT OK
Alleles are good
Alleles are bad
Spike
Mixture
Saturated
Poor Quality

Allele Comments
Right-click an allele in the *Electropherogram* or *Peak Table* and select *Edit Comments*. The *Edit Allele Comments* box appears. Select a comment from the *Comments Template* list or enter a new comment in the *Comments* field. Click **OK** and the comment will appear in the *Comments* column of the *Peak Table*. Only one user edited comment can be added to a peak. Comments automatically generated by the software cannot be removed. Additional user comments will simply be added next to the software comment.

View History
Opens the *Show Edit History* window. Shows a record of all manual edits performed on the peak. The *Show Edit History* window is only active when the *Help* → *User Management* → *Settings* → *Record Data Edit History* option is selected. **See Chapter 9 User Management**.

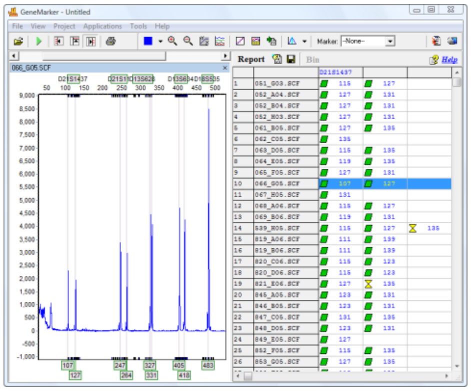
Report Table

The *Main Analysis* window *Report Table* contains additional information about sample peaks. Depending on the *Analysis Type* selected in the Run Wizard *Template Selection* box, the *Report Table* will contain appropriate information. **See Chapter 6 Reports and Printing**.

Navigation

The *Report Table* is linked to the other frames in the *Main Analysis* window. Double-click on the desired allele OR use the **Arrow keys** to move to the cell of interest and hit **Enter** key OR use **Alt + Arrow keys** to move to different cells and zoom in on the peak in the *Electropherogram*.

Select multiple cells by holding down **SHIFT** key OR hold left mouse button and drag over desired cells.

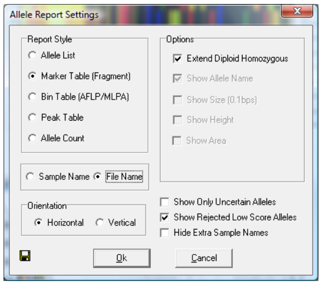


		D21S1437		
1	061_G03.SCF	🟩 115	🟩 127	
2	052_A04.SCF	🟩 127	🟩 131	
3	052_B04.SCF	🟩 127	🟩 131	
4	052_H03.SCF	🟩 127	🟩 131	
5	061_B05.SCF	🟩 127	🟩 135	
6	062_C05.SCF	🟩 135		
7	063_D05.SCF	🟩 115	🟩 135	
8	064_E05.SCF	🟩 119	🟩 135	
9	065_F05.SCF	🟩 127	🟩 131	
10	066_G05.SCF	🟩 107	🟩 127	
11	067_H05.SCF	🟩 131		
12	068_A06.SCF	🟩 115	🟩 127	
13	069_B06.SCF	🟩 119	🟩 131	
14	539_H05.SCF	🟩 115	🟩 127	⧗ 135
15	819_A06.SCF	🟩 111	🟩 139	
16	819_B06.SCF	🟩 111	🟩 139	
17	820_C06.SCF	🟩 115	🟩 123	
18	820_D06.SCF	🟩 115	🟩 123	
19	821_E06.SCF	🟩 127	⧗ 135	
20	845_A05.SCF	🟩 123	🟩 131	
21	846_B05.SCF	🟩 123	🟩 131	
22	847_C05.SCF	🟩 131	🟩 135	
23	848_D05.SCF	🟩 123	🟩 131	
24	849_E05.SCF	🟩 127		
25	852_F05.SCF	🟩 115	🟩 135	
26	853_G05.SCF	🟩 127	🟩 135	

The rules by which the *Report Table* and other frames in the *Main Analysis* window are linked are controlled by options in the *View → Preferences → Others* tab.

Display Settings

Click the **Report Settings** icon in the *Report Table* toolbar. The *Allele Report Settings* box will appear. Select different *Report Styles* to see additional options. After selecting *Report Style* options, click the **Save as Default** icon in the bottom left corner of the *Allele Report Settings* box. Your options will be saved and will be recalled the next time you select that *Report Style*. Additionally, select *View → Preferences → Others → Show Disabled Samples in Report* to include samples that are disabled in the *Sample File Tree*.



Report Style
○ Allele List
● Marker Table (Fragment)
○ Bin Table (AFLP/MLPA)
○ Peak Table
○ Allele Count

○ Sample Name ● File Name

Orientation
● Horizontal ○ Vertical

Options
☑ Extend Diploid Homozygous
☑ Show Allele Name
☐ Show Size (0.1bps)
☐ Show Height
☐ Show Area

☐ Show Only Uncertain Alleles
☑ Show Rejected Low Score Alleles
☐ Hide Extra Sample Names

Sort Options

Sort by Marker

Select *Sort by Marker* from the right-click menu and choose from the fly-out menu to sort *Ascending* or *Descending*. If *Ascending* is chosen, then low quality peaks will be sorted to the top of the table. If *Descending* is chosen, then the lower quality peaks will be placed at the bottom of the table. This option is only available with Marker Table and Allele Count *Report Styles*.

Sort by Column

Select *Sort by Column* from the right-click menu and choose from the fly-out menu to sort *Ascending* or *Descending*. If *Ascending* is chosen, then lesser values will be sorted to the top of the table and greater values to the bottom the table and vice versa if *Descending* is chosen. This option is available with all *Report Styles*.

Editing Peaks

To edit peaks, first left single or double-click the cell in the *Report Table* then right-click to see menu options or use **Hot Keys**.

Delete Peaks

Right-click the peak cell in the *Report Table* and select *Delete Peaks* (**Hot Key = DEL**). The deleted peak will be removed from the *Report Table*.

Confirm Peaks

If a peak is given a low quality score, it will receive a *Check* (yellow) or *Undetermined* (red) Quality ranking. To give the peak a *Pass* (green) Quality ranking, right-click the peak cell and select *Confirm Peaks* (**Hot Key = CTRL+M**). The peak will be marked *Pass* (green).

Peak Information

Hold down **CTRL** key and click the peak cell of interest. The *Allele Peak Info* box will appear containing information such as Sample, Dye, Size, Marker, Allele, Score and Comments. The information in these fields cannot be edited. This option is only available with Allele List, Marker Table, and Peak Table *Report Styles*.

Save Report Table

To save all information currently displayed in the *Report Table*, click the **Save Report** icon in the *Report Table* toolbar. Choose a directory, enter a filename (*_AlleleReport* is the default) and save as an Excel (.xls) or tab-delimited Text (.txt) file.

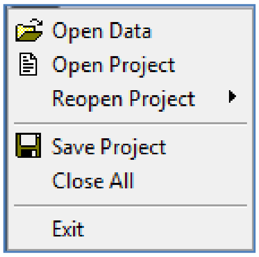
To export only selected cells in the report table, first select the cells by left-mouse drag across the cell range or hold **SHIFT** key and select cells. Right-click on the highlighted cells and select *Copy* (**Hot Key = CTRL+C**). The information is saved to the Windows clipboard and can be pasted into any common word processor or spreadsheet program like Microsoft Excel. The row and column headers for those cells will be copied with the highlighted cell information.

Menu Options

The following menu options can be found in the menu bar of the *Main Analysis* window.

File Menu

The *File* menu contains functions for opening and saving raw and processed data.



Open Data
Launches the *Open Data Files* window where the user can select raw data files for upload into GeneMarker. Accepted file formats include .fsa, .ab1, .abi, .scf, .rsd, .esd, .smd, .smr. **See Chapter 2 General Procedure.**

Open Project
Opens a folder search window where the user can select to open previously saved SoftGenetics *GeneMarker* project files (.sgf, .sfp)

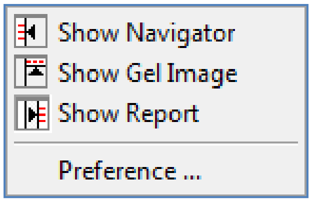
Re-Open Project
Saves the last four projects that were opened by *GeneMarker* and allows the user to launch any one of those four projects directly.

Save Project
Saves a SoftGenetics *GeneMarker* project (.sgf, .sfp) to a specified directory. Raw data files and analyzed data files with edits are saved within a project file. Starting with GeneMarker v. 1.96, the negative Y axis peaks of analyzed data are also saved in project files. Pull-down peaks may result from changes in optical alignment or polymer of the genetic analyzer.

Close All
Closes a project without exiting the program.
NOTE: It is recommended to select *Close All* before exiting the program.

Exit
Closes the *GeneMarker* program.

View Menu
The *View* menu contains options for how the data is displayed in the *Main Analysis* window.



Show Navigator/ Gel Image/ Report
Toggles the *Sample File Tree, Synthetic Gel Image*, or *Report Table* frames open and closed in the *Main Analysis* window.

Preference…
Activates the three-tab *Preferences* box.

Start up Settings
The *Start up Settings* tab, effective only at start up, allows you to select the *Run Method* and *General Settings*.

Run Method

Classic: Appropriate for experienced users. The user will move through the program data input, settings, and display options without prompting, by simply following the program's sequential analysis flow.

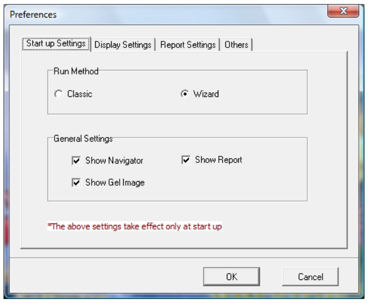
Wizard: Activates the Run Wizard which will guide the user through the program's operation. This setting is best for the inexperienced user.

General Settings

Show Navigator: When selected, the *Sample File Tree* will automatically be displayed in the *Main Analysis* window after data processing.

Show Gel Image: When selected, the *Synthetic Gel Image* will automatically be displayed in the *Main Analysis* window after data processing.

Show Report: When selected, the *Report Table* will automatically be displayed in the *Main Analysis* window after data processing.



Run Method
○ Classic ● Wizard

General Settings
☑ Show Navigator ☑ Show Report
☑ Show Gel Image

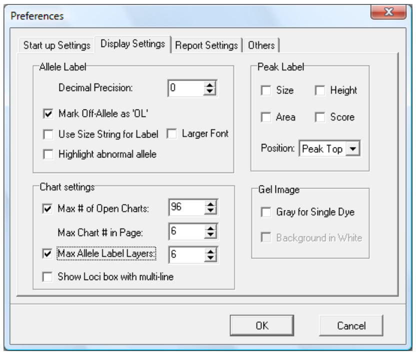
*The above settings take effect only at start up

Display Settings

The *Display Settings* tab is used to set how the data is displayed in the electropherograms.

Allele Label

Decimal Precision: Select 0 to 2 decimal places for peak size labeling.



Allele Label
Decimal Precision: 0
☑ Mark Off-Allele as 'OL'
☐ Use Size String for Label ☐ Larger Font
☐ Highlight abnormal allele

Peak Label
☐ Size ☐ Height
☐ Area ☐ Score
Position: Peak Top

Chart settings
☑ Max # of Open Charts: 96
Max Chart # in Page: 6
☑ Max Allele Label Layers: 6
☐ Show Loci box with multi-line

Gel Image
☐ Gray for Single Dye
☐ Background in White

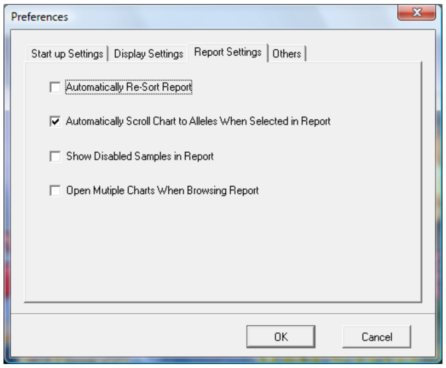
Mark Off-Allele as 'OL': Select this option to label alleles that are outside of allele ranges as 'OL'.
Use Size String for Label: Select this option to label peaks in the electropherograms according to size instead of the allele label. To display a rounded size string, set the *Decimal Precision* to 0.
Larger Font: Doubles the font size of the allele label characters. This increased font size will carry over to the *Print Report*.
Highlight abnormal allele: Select this option to highlight the labels of abnormal alleles with a bright color. Excellent for Abbott Cystic Fibrosis Kits or any chemistry that amplifies mutant and wild type alleles. Note: these alleles must be marked with a 1 in the Control Column of the Panel File. See Chapter 5 Panel Editor.

Chart Settings
Max # of Open Charts: Select the maximum number of samples you would like to display as an electropherogram at one time (Max = 96). Use the *Sample File Tree* right-click option *Select Max* to open the number of samples specified.
Max Chart # in Page: Select the maximum number of sample electropherograms you would like displayed in the *Main Analysis* window at one time (Max = 8). Use the *Sample File Tree* **PageUp/Down** option to select subsequent groups of samples.
Max Allele Label Layers: Select the number of allele label layers to view at once (Max = 10). This determines how far you must zoom in to clearly read neighboring allele labels and affects how the print report will be displayed. This setting also carries to the final reports in Special Applications such as MLPA.
Show Loci box with multi-line: Displays the loci boxes above the electropherogram (markers) staggered for clear view of the marker name when all multiple dyes are displayed at the same time in the electropherogram.

Peak Label
Choose up to four labels (size, height, area, score) to display as a flag next to individual peaks in the electropherogram.
Position: Choose to place the peak label at either the top of the peak, to the right side of the peak, or in the allele label in the *Electropherogram*.
Gel Image
Gray for Single Dye: When selected will display and print the gel image with a black background and white bands. When deselected the gel image will display a black background and colored bands (depending on dye color chosen to view).
NOTE: When all dye colors are selected, the bands in the gel image will be displayed in color regardless if this option is selected.
Background in White: Only available when *Gray for Single Dye* is selected. Will invert the gel image so that the background will be white and the band fragments will be black.

Report Settings

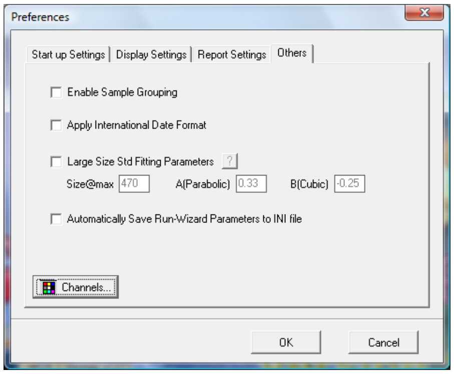
Automatically Re-Sort Report: Check this option if you would like GeneMarker to automatically re-sort the report every time you modify alleles. Un-check this feature if you want the report to remain sorted until you choose to re-sort.
Automatically Scroll Charts to Alleles When Selected in Report: You may choose whether to scroll to alleles in the trace when selecting the allele in the report. Leave this feature on to have the software automatically call up alleles in the trace when you double-click on them in the report.
Show Disabled Samples in Report: GeneMarker identifies samples that failed during electrophoresis or size calling. The default setting excludes the disabled samples from the report. The option may be selected to have failed or user-disabled samples to be identified in the report.



☐ Automatically Re-Sort Report
☑ Automatically Scroll Chart to Alleles When Selected in Report
☐ Show Disabled Samples in Report
☐ Open Mutiple Charts When Browsing Report

Open Multiple Charts When Browsing Report: When selected this option with keep all open charts active while the analyst is editing individual charts.

Others



Preferences

Start up Settings | Display Settings | Report Settings | Others

☐ Enable Sample Grouping

☐ Apply International Date Format

☐ Large Size Std Fitting Parameters ?

Size@max 470 A(Parabolic) 0.33 B(Cubic) -0.25

☐ Automatically Save Run-Wizard Parameters to INI file

Enable Sample Grouping: When *Project → Apply Sample Grouping* is implemented, the *Enable Sample Grouping* option will be automatically selected. De-select *Enable Sample Grouping* to inactivate the *Apply Sample Grouping* option. The *Apply Sample Grouping* information is saved and can be recalled by selecting *Enable Sample Grouping*. **See Chapter 8 Additional Tools – Filename Group Tool.**

Apply International Date Format: Check this option if you would like the dates of the Reports for Applications to be in the International format day/month/year.

Large Size Std Fitting Parameters: Customizes the Large Size Call Algorithm by applying the user specified values to the Large Size Call. See Chapter 2 General Procedure The ? icon links users to a spread sheet that assists in determining the best parameters for the custom large size standard.

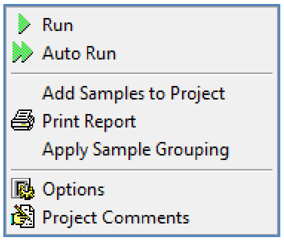
Automatically Save Run Wizard Parameters: Saves Run Wizard parameters in an .ini file ProjectName_RunWizardParameters.ini

Channels: Opens the *Set Channels* box and allows the user to choose from ABI, MegaBACE, and Beckman-Coulter standard dye colors. The user can also manually enter dye color and name. The default channel color setup is ABI. **See Chapter 2 General Procedure**.

Project Menu

The *Project* menu contains options for how the data is processed and printed.

Run



Activates the Run Wizard and begins the data processing setup. This allows the user to select or adjust program settings in a sequential manner. The same process action can also be accomplished by clicking the *Run* icon in the toolbar.

Auto Run

GeneMarker will process data using the last set of parameters selected. If one or more of the parameters require changing to improve analysis, select *Project → Options*, change the desired setting(s), and re-process the samples for analysis.

Add Samples to Project

The user can add samples to a project that has already been sized and analyzed. When selected, the *Open Data Files* box will appear. Click **Add** to select individual files to the project and click **OK**. The raw data file will be sized and processed with the same settings as the other files in the project and added to the bottom of the *Sample File Tree*.

Print Report

Selecting *Print Report* launches the *Print Report Settings* box which allows the user to define display settings in the *Print Report*. The software permits printing of the sample electropherograms. You can choose to print all samples, selected samples, or print samples along with the allele table, if desired. **See Chapter 6 Reports and Printing.**

Options

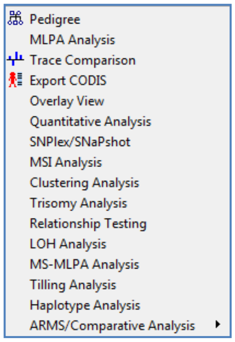
Allows you to access and change parameters in the *Project Option Settings* window. This three-tab window contains settings identical to the Run Wizard. Adjust settings in the *Project Options Settings* box *before* selecting Auto Run. **See Chapter 2 General Procedure.**

NOTE: Auto Run does not need to be selected after adjusting the *Additional → Allele Evaluation → Peak Score* settings. The changes will automatically be applied when the *Project Option Settings* window is closed.

Project Comments

Allows the user to write free-form comments regarding the analysis. These comments are saved with the project file and can be displayed in the *Print Report.*

Applications Menu



The *Applications* menu contains individual modules for specific data and analysis types. These modules present advanced features and reporting options necessary for the particular application. **See Chapter 7 Special Applications** for more information on each analysis application.

Pedigree

Display and check genotype calls using a pedigree chart. Data must be run with the correct size standard and Panel prior to using the *Pedigree* function.

MLPA Analysis

Analyze data from Multiplex Ligation-dependent Probe Amplification (MLPA). Offers two normalization analysis methods and includes a customizable patient report for clinical use.

Trace Comparison

Used with AFLP data, the *Trace Comparison* module was designed to identify length polymorphisms between closely-related species. **See Chapter 7 Special Applications – AFLP.**

Export CODIS

Developed for forensic scientists analyzing short tandem repeat fragment data. Exports the CMF 3.0 (.xml) and CMF 1.0 (.dat) files for upload into the FBI's CODIS database. **See Chapter 7 Special Applications – HID.**

Overlay View

Allows the user to graphically display any combination of samples and dye colors. This feature includes a 2-Dimensional and a 3-Dimensional view of the selected samples. **See Chapter 8 Additional Tools.**

Quantitative Analysis

Offers two different analysis types for quantifying peak areas: *Curtain Method* and *De-convolution Method.*

SNPlex/SNaPshot

A SNP discovery application for use with SNPlex, SNaPshot, and SNuPe data analysis. Includes two color comparison and a ratio plot of homozygotes and heterozygotes.

MSI Analysis

Allows comparison of microsatellite markers in normal and tumor samples to detect instability. Includes a trace comparison histogram and a patient report with positive and negative MSI marker calls.

Clustering Analysis

Often used with AFLP data, the *Clustering Analysis* module creates a dendrogram view of all samples in a project. Users can choose from *Euclidean Distance* or *Pearson Correlation* and *Single, Complete*, and *Linkage* type analyses. **See Chapter 7 Special Applications – AFLP.**

Trisomy Analysis

Detects additional alleles beyond the expected ploidy within a marker by peak intensity or peak area and calculates a confidence score. A patient report is also available

Relationship Testing

Uses Identity by Descent methods to search a database for nearest relatives, kinship analysis and parentage verification. Pedigree drawing may also be helpful in reviewing uniparental disomy.

LOH Analysis

The user defines limits for a ratio plot which then uses normalized peak intensities or peak areas to determine the presence or absence of alleles compared to a reference trace. The reference and sample traces are overlaid for easy LOH identification.

MS-MLPA Analysis

Detects methylation sites within promoter regions and for genomic imprinting applications

TILLING

Detects SNPs generated by the TILLING technique. By using the internal size standard to align each capillary and generating a reference trace from all of the samples in the run, the reference can be subtracted from each individual sample trace yielding a plot highlighting the SNPs. Additionally, a table shows what the expected size of the complementary fragment should be to determine if each peak is a true variation.

Haplotype Analysis

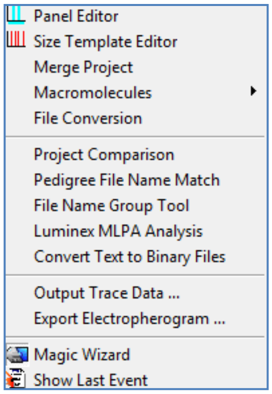
The program uses the allele calls of children and parents to assign phase of the alleles with a first order approximation.

ARMS/Comparitive Analysis

Allows comparison of pairs of files for a given individual; includes electropherograms, trace comparison and allele report in a patient report format.

Tools Menu

The *Tools* menu contains the *Panel* and *Size Editors* in addition to other miscellaneous modules.



Panel Editor

Provides a variety of tools to adjust, edit, and create control Panels. **See Chapter 5 Panel Editor**.

Size Template Editor

Allows the comparison of sample files against a selected size standard, to modify and save the size standard for future use, or create a customized size standard. **See Chapter 4 Fragment Sizing Standards**.

Pedigree File Name Match

Allows the user to automatically add additional files to a previously created pedigree tree. A .smp file is exported. **See Chapter 7 Special Applications – Pedigree Chart.**

Merge Project

Combines the individual file results from different projects (multiplexes) into one complete genotype report that may be saved to print out separately or imported into Cluster Analysis or Relationship Testing.

Macromolecules

Aligns the results for a project without a size standard. Uses fragments that are consistent from one lane to another to correct for migration differences from one capillary to another.

File Conversion

To combine data for X and Y axis from from unique instruments from a single file (X,Y_file) or two files (X_file, Y_file).

File Name Group Tool

Used specifically with the MSI and LOH applications, the *Filename Group Tool* allows users to define how reference samples and tumor samples should be grouped or paired. A Text (.txt) file is exported. **See Chapter 8 Additional Tools.**

Output Trace Data

Provides the option to output the raw or sized trace data as a TXT or SCF file. Select the samples to include, dye colors, data type, and the directory to output the trace files. **See Chapter 8 Additional Tools.**

Luminex MLPA Analysis

For researchers and clinicians using Luminex colored bead method for MLPA analysis. **See Chapter 7 Special Applications – MLPA.**

Project Comparison

Allows the user to compare the same data set (two different projects) and detect differences based on a number of parameters including peak size and height, quality score, and commented alleles. **See Chapter 8 Additional Tools.**

Convert Text to Binary Files

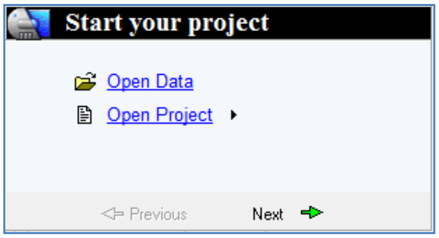
For customers developing their own instrumentation, the *Convert Text to Binary Files* option allows users to upload four or five-color Text files (without headers) for conversion into SCF (four-color data) or SG1 (five-color data) trace files for analysis with *GeneMarker*. **See Chapter 8 Additional Tools.**

Export Electropherogram

Allows the user to export the trace images to a specified folder (*.png, *.bmp or *.jpeg).

Magic Wizard

Contains three option boxes: *Start Your Project, Run* and *Report.*



Start Your Project

Allows the user to easily access the *Open Data* or *Open Project* upload windows. The user can also re-open the four previously opened projects by selecting the black arrow next to *Open Project.*

Run

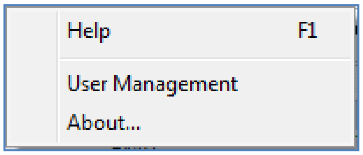
Selecting *Run* launches the Run Wizard. Selecting *AutoRun* will process the data automatically with the process options currently selected. **See Chapter 2 General Procedure.**

Report

Allows the user to *Save Project* or *Print Report*. Selecting *Print Report* will launch the *Print Report Settings* box. **See Chapter 6 Reports and Printing.**

Show Last Event

Opens the last active *Data Process* action.



Help Menu

Help

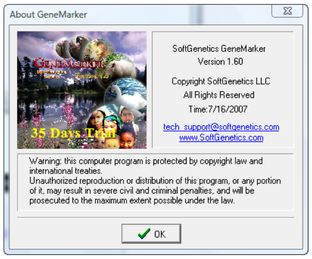
Launches a searchable version of this manual.

User Management

Allows an administrator to assign access rights to different users. Also used to set up the password protection feature. Information from User Management is used to populate the header of some of the specialized application reports. **See Chapter 9 User Management**.

About…

Displays information specific to the version of GeneMarker running on the computer. Also contains links to email Technical Support and the SoftGenetics website.



SoftGenetics GeneMarker
Version 1.60
Copyright SoftGenetics LLC
All Rights Reserved
Time:7/16/2007
tech_support@softgenetics.com
www.SoftGenetics.com

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Unauthorized reproduction or distribution of this program, or any portion of it, may result in severe civil and criminal penalties, and will be prosecuted to the maximum extent possible under the law.

Main Toolbar Icons

Open Data
Opens data input dialog box to begin analysis.

Run Project
Opens Run Wizard for processing the data.

Show/Hide Toggles
Displays or hides the *Sample File Tree, Synthetic Gel Image*, and *Report Table* frames, respectively.

Print Report
Provides the user display options for the *Print Report*.

Show Color
Allows the user to select all colors to view, hide all colors, or choose a single dye layer. Choose a single dye by single left mouse clicking on the icon.

Zoom In
Use the icon to zoom in on the image, or hold down the left mouse button and draw a box, from the top left corner to bottom right corner, around the area you wish to zoom in.

Zoom Out
Use the icon to zoom out on the image, or hold down the left mouse button and draw a box, from the bottom right corner to top left corner.

Set Axis
The default setting automatically sets the Y-axis according to the maximum peak intensity of the samples. Two other options are available: auto fit the Y-axis using peak intensities of the alleles, or the user can select the ranges for the X- and Y-axis.

Browse by All Colors
Displays a comparative view of sample electropherograms by dye color. Individual samples can be selected from the drop-down menu.

Allele Call Icons

These icons are only available after the raw data has been processed and the *Sample File Tree Allele Call* folder is selected.

Size Calibration
Displays calibration charts for linearity of lane analysis.

Show Chart/Table
Toggles display to show only the *Peak Table*, the *Peak Table* and *Electropherogram*, or just the *Electropherogram*.

Save Peak Table
Exports the *Peak Table* as an Excel (.xls) file or tab-delimited Text (.txt) file.

Call Allele
Call alleles by sample(s), by marker, or by dyes. Permits slight modifications to the samples without having to activate Run Wizard again. Settings to change include *Peak Detection Threshold, Stutter Peak Filter*, and *Peak Score Threshold*.

Marker:
Yellow_1

Marker Drop-down Menu
Allows the selection of a marker to view. This is available after the samples have been compared to a Panel.

Event Log
Displays each lane's processing success or failure.

Magic Wizard
Activates the *Start Your Project, Run* and/or *Report* dialog boxes.

Report Table Icons

The icons are located directly above the *Report Table*.

Report Settings
Allows the user to customize *Report Table* display settings.

Save Report
Exports the *Report Table* as an Excel (.xls) file or tab-delimited Text (.txt) file.

Bin
Customize Bin Column
Allows the user to select which bins to include/exclude in the *Report Table*.

Chapter 4 Fragment Sizing Standards

Chapter 4 Fragment Sizing Standards

Size Template Editor
Size Calibration Charts

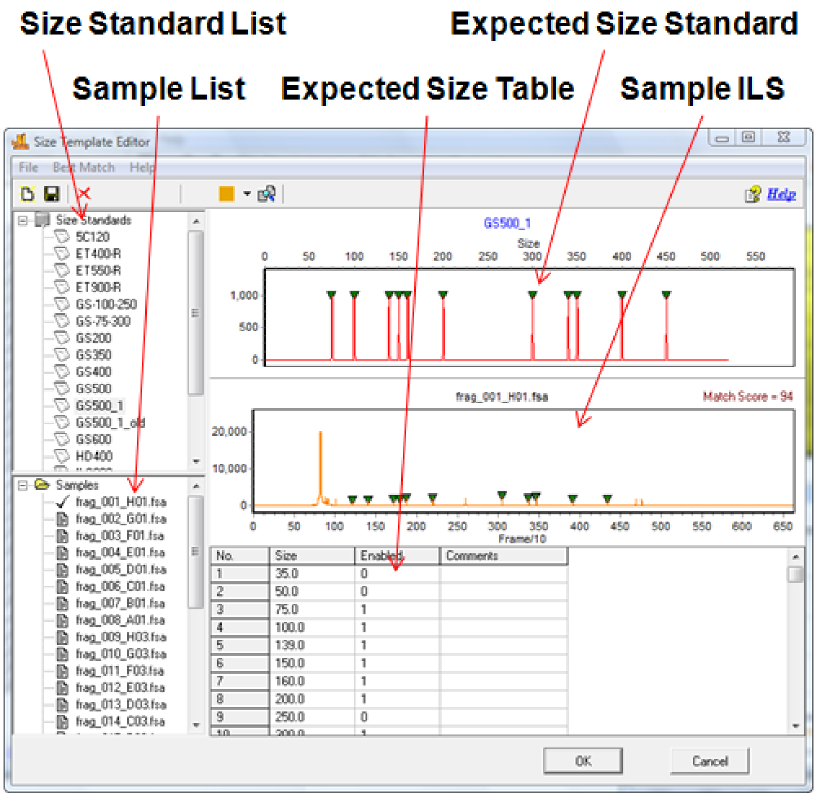
Size Template Editor

The *Size Template Editor* is a tool in GeneMarker for creating and modifying Size Standards. To open the *Size Template Editor*, select *Tools → Size Template Editor* from the menu bar OR click the **Size Template Editor** icon in the Run Wizard *Template Selection* box.

Due to differential fragment mobility in capillary gel electrophoresis, a sizing standard must be applied. Each sample run through a CE instrument will contain an Internal Lane Standard (ILS). The ILS contains peaks of known size and is usually tagged with red or orange fluorescent dye. Since the ILS dye-labeled fragments migrate through the same capillary as the other dye-labeled sample fragments, they are subject to the same environmental conditions and can therefore be used as a guide to determine the size of the other fragments in the sample. A Size Standard template is applied to each ILS and sizes between the known ILS peaks are interpolated.

NOTE: GeneMarker is optimized to size fragments with linear mobility. Larger fragments or those run through a high viscosity gel (i.e. agarose) do not migrate linearly and therefore cannot be analyzed with GeneMarker at this time.

Size Template Editor


Size Standard List
Expected Size Standard
Sample List
Expected Size Table
Sample ILS
Size Template Editor
File
Best Match
Help
Help
Size Standards
5C120
ET400-R
ET550-R
ET900-R
GS-100-250
GS-75-300
GS200
GS350
GS400
GS500
GS500_1
GS500_1_old
GS600
HD400
Samples
frag_001_H01.fsa
frag_002_G01.fsa
frag_003_F01.fsa
frag_004_E01.fsa
frag_005_D01.fsa
frag_006_C01.fsa
frag_007_B01.fsa
frag_008_A01.fsa
frag_009_H03.fsa
frag_010_G03.fsa
frag_011_F03.fsa
frag_012_E03.fsa
frag_013_D03.fsa
frag_014_C03.fsa
GS500_1
Size
0 50 100 150 200 250 300 350 400 450 500 550
1,000
500
0
frag_001_H01.fsa
Match Score = 94
20,000
10,000
0
0 50 100 150 200 250 300 350 400 450 500 550 600 650
Frame/10
OK
Cancel

No.	Size	Enabled	Comments
1	35.0	0	
2	50.0	0	
3	75.0	1	
4	100.0	1	
5	139.0	1	
6	150.0	1	
7	160.0	1	
8	200.0	1	
9	250.0	0	

Size Standard List

The *Size Standard List* contains all pre-defined Size Standards and any custom-made Size Standards. Single-left click a Size Standard in the list to select it. The *Expected Size Standard* trace and *Size Table* will appear on the right.

Additional Options

To see additional options for each Size Standard, right-click the Size Standard name and the right-click menu will appear with the following options.

Delete Size Standard
Select *Delete* to delete the Size Standard from the *Size Standard List* and from the SoftGenetics GeneMarker Size Standard directory.
NOTE: This action is irreversible.

Export Size Standard
Opens the *Save As* window. Choose a directory folder and click **Save**. The Size Standard will be copied to the selected directory and will also remain in the *Size Standard List* and SoftGenetics GeneMarker Size Standard directory. The Size Standard will be exported as an XML file which can be opened with Internet Explorer, Microsoft Excel, or Notepad.

Reload Size Standard
Click *Reload* to undo editing changes to the Size Standard. The most recently saved Size Standard will be restored.
NOTE: If the user selects **Save** Size Standard and then answers "NO" to the "Size Standard has been changed, save changes?" the changes will remain in the *Expected Size Standard* and *Size Table* until the user chooses *Reload* or GeneMarker program is closed.

Sample List

The *Sample List* contains a list of all the samples in the dataset. Double-click the filename and the sample's ILS trace will appear in the *Sample ILS* frame. Use the **Up/Down Arrow** keys to scroll through samples in the list.

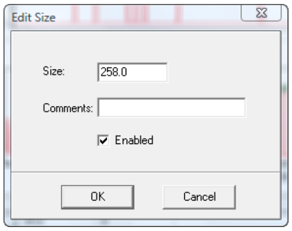
Expected Size Standard and Size Table

The *Expected Size Standard* frame displays, as a trace, all the known fragment peaks that are expected to appear in the *Sample ILS*. Single left-click a green triangle atop a peak to select the peak. The green triangle will turn yellow when the peak is selected.

Additional Options

Once a peak is selected, right-click anywhere in the *Expected Size Standard* frame. The right-click menu will appear with the following options.

Edit Size
The *Edit Size* box appears. Adjust parameters and click **OK**.
Size: Enter the expected basepair size of the ILS fragment.
Comments: Enter free form text regarding the Size.
Enabled: When selected, a "1" will appear in the *Expected Size Table*. Deselect this option to disable the Size in the Size Standard. Disabled sizes will be used for pattern recognition in the sample ILS but will not be used to size fragments in the other dye colors. Disable a Size if its position is variable from sample to sample.



Size: 258.0
Comments:
☑ Enabled

NOTE: If the *Enabled* value is changed in the *Size Table*, you must click another cell in the *Size Table* before saving the Size Standard or the change will not take effect.

Insert Size
Right-click at the position in the *Expected Size Standard* frame or in the *Sample ILS* where the Size should be placed. The *Edit Size* box will appear. GeneMarker will automatically interpolate the value in the *Size* field if there are two or more Sizes present in the trace. Adjust as necessary and click **OK**. A green triangle will appear at the cursor position indicating where the new Size was placed.
NOTE: The height of the new Size in the *Expected Size Standard* trace is dependent on the height of the peak in the corresponding *Sample ILS* trace.

Delete Size
Select *Delete Size* to remove the Size completely from the Size Standard. Alternatively, the Size can be disabled by deselecting *Enabled* in the *Edit Size* box or by placing a "0" in the *Enabled* column of the *Expected Size Table*.
NOTE: Sizing is often more successful when there are many Sizes in the Size Standard.

Set Value to Column
Makes all values in the column equal to the value in the highlighted cell. Only available in the *Expected Size Table*.

Sample ILS

The *Sample ILS* frame displays the selected sample's ILS trace. Click the **Show Dye** icon in the toolbar to cycle through the other dye colors. Right-click at a peak without a green triangle indicator and choose *Insert Size*. The *Edit Size* box will appear. Adjust as necessary and click **OK**. The green triangle will now appear atop the peak and also in the *Expected Size Standard*.

Match Score

Appears in the upper right corner of the *Sample ILS* and corresponds to the degree of pattern match between the sample's ILS and the Size Standard selected. Perfect matches receive a score of 100, no correlation receives a score of 0.

Navigation in the *Sample ILS* frame is similar to the navigation options in the *Main Analysis* window. **See Chapter 3 Main Analysis Overview.**

Procedure

As mentioned previously, Size Standards are created to assign basepair size information to fragment peaks in a sample ILS. The other dye color fragment peak positions are then interpolated based on a linear size scale from the basepair sizes assigned to the peaks in the ILS. GeneMarker's *Size Template Editor* tool allows users to apply pre-defined commercial Size Standards or create new custom Size Standards based on the dataset ILSs.

Pre-defined Size Standards include:

5C120
ET400-R
ET550-R
ET900-R
GS-100-250
GS-75-300
GS200
GS350
GS400
GS500
GS500_1
HD400
ILS500
ILS600
Liz120
Rox1000
SEQ_600
SNPlex_48plex_v1

Pre-Defined Size Standards

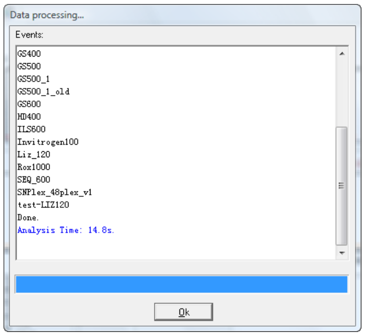
There are two ways to choose a pre-defined Size Standard for the dataset. If the Size Standard name is known, simply single left-click the Size Standard name in the *Size Standard List* and click **OK** in *Size Template Editor*. The selected Size Standard will then appear in the *Size Standard* field of Run Wizard *Template Selection* box and will be used to size the data.

Alternatively, if the Size Standard name is not known, follow the *Best Match* steps below.

1. In *Size Template Editor*, select *BestMatch* → *Match All*
2. The *Data Processing* box appears
3. GeneMarker cycles through all Size Standards
4. Click **OK** when *Data Process* is finished
5. The Size Standard with the best average *Match Score* across all samples in the dataset will be highlighted in the *Size Standard List* and appear in the *Expected Size Standard* frame

NOTE: *BestMatch* will not always choose the correct Size Standard. User inspection is required.

6. Once the Size Standard is chosen, click **OK** in the *Size Template Editor*
7. The selected Size Standard will then appear in the *Size Standard* field of Run Wizard *Template Selection* box and will be used to size the data.

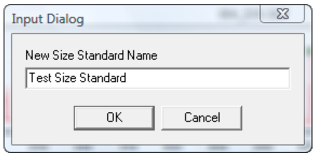


```
GS400
GS500
GS500_1
GS500_1_old
GS600
HD400
ILS600
Invitrogen100
Liz_120
Rox1000
SEQ_600
SNPlex_48plex_v1
test-LIZ120
Done.
Analysis Time: 14.8s.
```

Custom Size Standard Creation

Follow the steps below to create a new Size Standard based on the dataset currently uploaded to GeneMarker.

1. In *Size Template Editor*, select *File* → *New Size Standard* <u>*OR*</u> click the **New Size Standard** icon
2. The *Input Dialog* box appears
3. Enter a Size Standard name and click **OK**

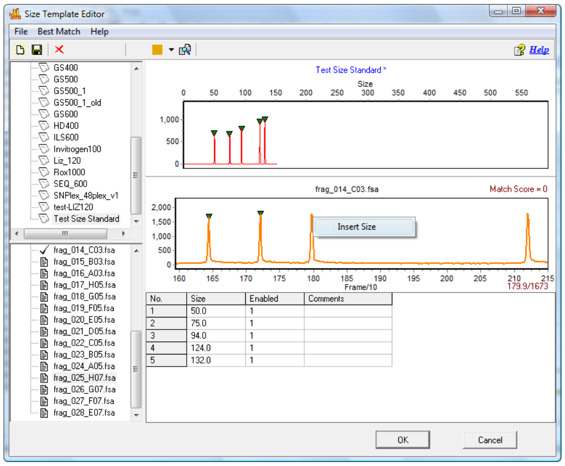


New Size Standard Name: Test Size Standard

4. The *Expected Size Standard* frame will be blank
5. Right-click at a known peak in the *Sample ILS* frame
6. Select *Insert Size*
7. The *Insert Size* box appears
8. Enter the basepair size of the peak in the *Size* field and click **OK**
9. A green triangle will appear atop the peak in the *Sample ILS* and a new peak will appear in the *Expected Size Standard* frame
10. Continue *Insert Size* operation for the rest of the peaks in the *Sample ILS*
11. GeneMarker will interpolate Size values after two peaks are added to the Size Standard

NOTE: It is recommended to use the interpolated Size values when creating a Size Standard due to the differential migration patterns of each sample.

12. When the Size Standard is complete, select *File → Save Changes* OR click **Save Changes** icon
13. Click **OK** in *Size Template Editor*
14. Proceed with Run Wizard data analysis


Size Template Editor
File
Best Match
Help
Help
GS400
GS500
GS500_1
GS500_1_old
GS600
HD400
ILS600
Invitrogen100
Liz_120
Rox1000
SEQ_600
SNPlex_48plex_v1
test-LIZ120
Test Size Standard
frag_014_C03.fsa
frag_015_B03.fsa
frag_016_A03.fsa
frag_017_H05.fsa
frag_018_G05.fsa
frag_019_F05.fsa
frag_020_E05.fsa
frag_021_D05.fsa
frag_022_C05.fsa
frag_023_B05.fsa
frag_024_A05.fsa
frag_025_H07.fsa
frag_026_G07.fsa
frag_027_F07.fsa
frag_028_E07.fsa
Test Size Standard *
Size
0
50
100
150
200
250
300
350
400
450
500
550
1,000
500
0
frag_014_C03.fsa
Match Score = 0
2,000
1,500
1,000
500
0
Insert Size
160
165
170
175
180
185
190
195
200
205
210
215
Frame/10
179.9/1673
OK
Cancel

No.	Size	Enabled	Comments
1	50.0	1	
2	75.0	1	
3	94.0	1	
4	124.0	1	
5	132.0	1	

Icons and Functions

The following are explanations of menu and icon options in *Size Template Editor*.

Menu Options

The *Size Template Editor* contains three menu options – *File, BestMatch*, and *Help*. The *File* menu allows the user to create, save, and export Size Standards. The *BestMatch* menu contains options for selecting a Size Standard. The *Help* menu shows navigation hints for *Size Template Editor*.

File Menu

New Size Standard

Opens the *Input Dialog* box with a field to enter a new Size Standard name. Follow the steps above – *Custom Size Standard Creation.*

Delete Current Size Standard

Deletes the Size Standard that is currently highlighted in the *Size Standard List*

NOTE: This action is irreversible.

Save Changes

Saves edits and changes to the Size Standard in the SoftGenetics GeneMarker Size Standard directory

Save as New Size Standard

Opens the *Input Dialog* box with a field to enter a new Size Standard name. The Size Standard is added to the *Size Standard List* and saved in the SoftGenetics GeneMarker Size Standard directory.

Import Size Standard

Opens a Windows Explorer window to the SoftGenetics GeneMarker Size Standard directory. Use the *Import Size Standard* option to find previously exported Size Standard Files (.xml) on local or networked computers.

Export Size Standard

Exports the currently selected Size Standard in the *Size Standard List* as an XML file to a specified directory on a local or network computer.

Import ABI Size Standard

Opens a Windows Explorer window to the same folder the sample files were uploaded from.

Export ABI Size Standard

Exports the currently selected Size Standard in the *Size Standard List* as an XML file to a specified directory on a local or network computer.

Exit
Closes the *Size Standard Editor* tool. Be sure to save changes to the Size Standard before exiting.

BestMatch

Match Selected
When selected, the *Data Process* box appears. Each sample in the dataset is compared to the currently highlighted Size Standard in the *Size Standard List*. The green triangle indicators are adjusted to give the best match possible.

Match All
When selected, the *Data Process* box appears. All samples in the dataset are compared to each Size Standard. The *Match Scores* for each sample are averaged together. The Size Standard with the highest average *Match Score* for the dataset is chosen as the *Best Match*.

Help

The *Help* menu contains a link to *Hot Keys* in *Size Template Editor*. Click *Hot Keys* and the *Size Editor Action Help* box appears.

Toolbar Icons

Size Template Editor
Found in the Run Wizard *Template Selection* box or in the *Tools* menu.

Create New Size Standard
Opens the *Input Dialog* box with a field to enter a new Size Standard name. Allows for the creation of a new Size Standard.

Save Changes
Saves modifications made to the Size Standard to the SoftGenetics GeneMarker Size Standard directory.

Delete
Deletes the Size Standard that is currently highlighted in the *Size Standard List*.
NOTE: This action is irreversible.

Show Dye
Allows the user to select a single dye color to view in the *Sample ILS* frame. Cycle through the colors by left-clicking the icon or use the drop-down menu.

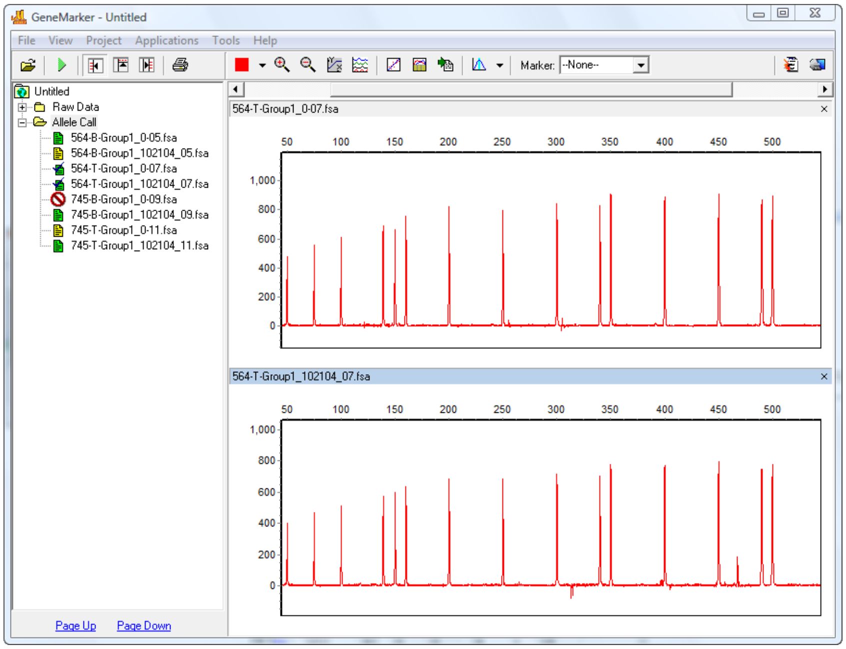
Size Match
Automatically places the green size marker triangles atop the peaks of the sample trace and matches it with the selected Size Standard.

What to Expect

Once the Size Standard is created, it can be applied to the dataset. **Save** the edited Size Standard in *Size Template Editor* then exit *Size Template Editor*. If the *Size Template Editor* was accessed via the Run Wizard *Template Selection* box icon then the selected Size Standard will appear in the *Size Standard* field. If the *Size Template Editor* was accessed via the *Tools* menu then click the **Run Process** icon in the *Main Analysis* toolbar. The Run Wizard will appear. Select the Size Standard from the *Size Standard* drop-down menu in the Run Wizard *Template Selection* box. Proceed through the other Run Wizard boxes and click **OK** when the *Data Process* window is complete. The Size Standard will be applied.

The success of size calling for each sample is indicated by the green, yellow, and red sheet next to the sample filename in the *Sample File Tree* of the *Main Analysis* window. The lane sizing quality is determined by the *Match Score* which in turn is a calculation of how closely the sample's ILS peaks match to the selected Size Standard. If a sample receives a low *Match Score*, the sample will be marked with a yellow sheet. If the size calling failed (the sample's ILS peaks could not be aligned with the Size Standard selected) then the sample will be marked with a red strike-through. When low score or failed samples occur, select the **Size Calibration Charts** icon in the main toolbar to correct the size calling.

Low Match Score and Failed Samples


GeneMarker - Untitled
File View Project Applications Tools Help
Marker: --None--
Untitled
Raw Data
Allele Call
564-B-Group1_0-05.fsa
564-B-Group1_102104_05.fsa
564-T-Group1_0-07.fsa
564-T-Group1_102104_07.fsa
745-B-Group1_0-09.fsa
745-B-Group1_102104_09.fsa
745-T-Group1_0-11.fsa
745-T-Group1_102104_11.fsa
Page Up
Page Down
564-T-Group1_0-07.fsa
564-T-Group1_102104_07.fsa
50 100 150 200 250 300 350 400 450 500
1,000
800
600
400
200
0

Size Calibration Charts

The *Size Calibration Charts* tool is designed to aid the user in determining success or failure of size call after GeneMarker's automatic sizing is performed. Click the **Size Calibration Charts** icon in the main toolbar of the *Main Analysis* window. As mentioned previously, once a Size Standard has been applied to the dataset, *Size Match Score* indicators appear next to the filename in the *Main Analysis* window *Sample File Tree*. Samples with a high *Match Score* are indicated by a green sheet; those with a low *Match Score* have a yellow sheet. Samples where size calling failed receive a red strike-through. To analyze how each individual sample was matched to the Size Standard selected, access the *Size Calibration Charts*. Within *Size Calibration Charts*, the user can modify how each sample was sized and view the statistical information for disabled Size Standard peaks.

Sample List

The *Sample List* includes filename, *Match Score*, and disabled peak information for each sample in the dataset. Sort the list by single left-clicking the column header. The list will re-sort in ascending or descending order based on the values in the column selected. Single left-click a sample to view its *Sample ILS* and *Calibration Plots* on the right OR use the **Up/Down Arrow** keys. Right-click the sample row and select *Mark as Failed* to disable the sample; select *Unmark Failed* to reverse the action. Disabled samples will appear "grayed-out" in the *Sample List*.

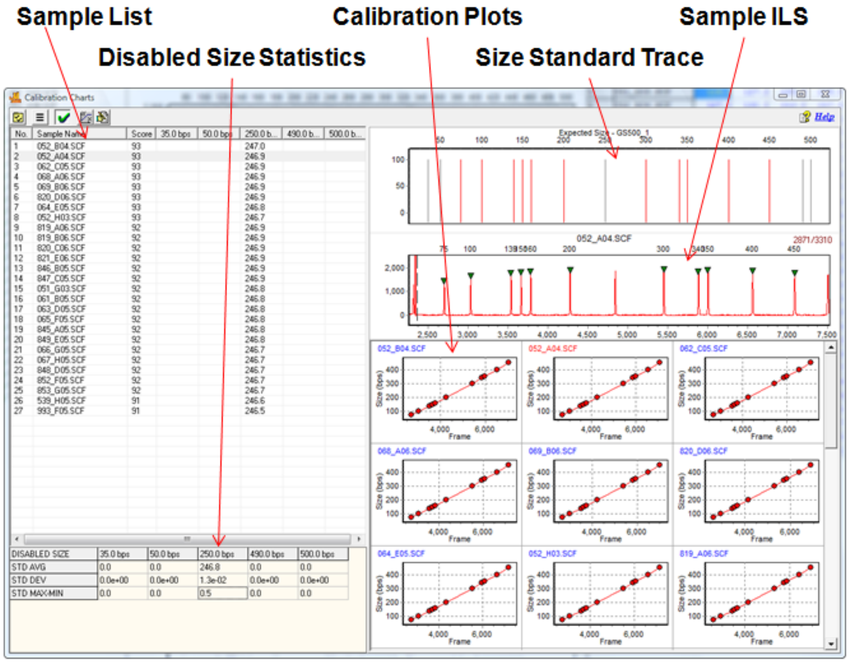
Score

The *Score* column displays the sample's *Match Score* which corresponds to the degree of pattern match between the sample's ILS and the Size Standard selected. Perfect matches receive a score of 100; no correlation receives a score of 0 and the sample is considered to have failed size calling.

Disabled Size Columns

The Sizes that were disabled in the Size Standard (see *Size Template Editor* section above) will appear as column headers in the *Sample List*. If no Sizes were disabled then only the *Sample Name* and *Score* columns will appear in the *Sample List*. The basepair size position of the disabled peak is reported for each sample. If the disabled peak is at the beginning or end of the Size Standard, no basepair size position will reported.

Size Calibration Charts



No.	Sample Name	Score	35.0 bps	50.0 bps	250.0 b...	490.0 b...	500.0 b...
1	052_B04.SCF	93			247.0		
2	052_A04.SCF	93			246.9		
3	062_C05.SCF	93			246.9		
4	068_A06.SCF	93			246.9		
5	069_B06.SCF	93			246.9		
6	820_D06.SCF	93			246.9		
7	064_E05.SCF	93			246.8		
8	052_H03.SCF	93			246.7		
9	819_A06.SCF	92			246.9		
10	819_B06.SCF	92			246.9		
11	820_C06.SCF	92			246.9		
12	821_E06.SCF	92			246.9		
13	846_B05.SCF	92			246.9		
14	847_C05.SCF	92			246.9		
15	051_G03.SCF	92			246.8		
16	061_B05.SCF	92			246.8		
17	063_D05.SCF	92			246.8		
18	065_F05.SCF	92			246.8		
19	845_A05.SCF	92			246.8		
20	849_E05.SCF	92			246.8		
21	066_G05.SCF	92			246.7		
22	067_H05.SCF	92			246.7		
23	848_D05.SCF	92			246.7		
24	852_F05.SCF	92			246.7		
25	853_G05.SCF	92			246.7		
26	539_H05.SCF	91			246.6		
27	993_F05.SCF	91			246.5		

DISABLED SIZE	35.0 bps	50.0 bps	250.0 bps	490.0 bps	500.0 bps
STD AVG	0.0	0.0	246.8	0.0	0.0
STD DEV	0.0e+00	0.0e+00	1.3e-02	0.0e+00	0.0e+00
STD MAX-MIN	0.0	0.0	0.5	0.0	0.0

Disabled Size Statistics

If Sizes were disabled in the Size Standard (see previous section – *Size Template Editor*), then the *Disabled Size Statistics* table will appear in the bottom left corner of the *Size Calibration Charts* window. The average basepair position, the standard deviation, and the difference between the maximum and minimum basepair positions across all samples are calculated for each ILS peak matched to the disabled peak's position. No statistics will be calculated for disabled peaks at the beginning or end of the Size Standard.

Size Standard Trace

The *Size Standard Trace* displays a synthetic trace of the selected Size Standard. Enabled Sizes are red; disabled Sizes are grey. Each peak in the *Size Standard Trace* represents the expected basepair size of peaks in the sample ILS.

Sample ILS

The *Sample ILS* displays the currently selected sample's ILS trace. Single left-click samples in the *Sample List* to see additional samples OR use the **Up/Down Arrow** keys. The green triangle peak indicators appear atop peaks that correspond to the enabled Sizes in the *Size Standard Trace*. The basepair size associated with the green triangle peak indicator is located above the electropherogram. The peaks selected for size calling can be edited in the *Sample ILS* frame as described below.

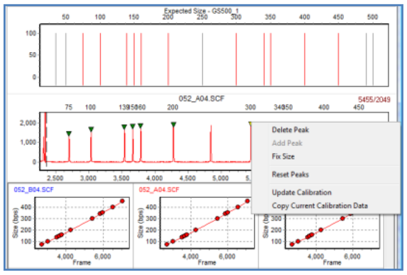
Navigation in the *Sample ILS* frame is similar to navigation options in the *Main Analysis* window. **See Chapter 3 Main Analysis Overview**.

Editing Size Call

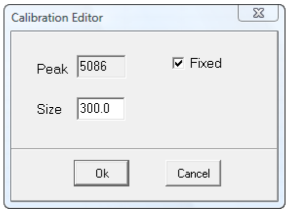
Single left-click a green triangle peak indicator to select it. The triangle that is currently selected will be yellow. To move the green triangle, hold down the **CTRL** key and left-click and drag it to the desired position. Right-click the green triangle peak indicator or right-click the top of an unmarked peak to see additional options.

Delete Peak
Removes the green triangle peak indicator from the *Sample ILS* and the peak will not be considered in the *Match Score* calculation. The *Match Score* calculation is updated when *Update Calibration* is selected.

Add Peak
Right-click at the peak position and select *Add Peak*. A green triangle peak indicator will appear at the cursor position. To move the green triangle hold down the **CTRL** key and left-click and drag it to the desired position. The newly added peak will be included in the *Match Score* calculation. The *Match Score* calculation is updated when *Update Calibration* is selected.
NOTE: *Add Peak* is only available when no other green triangle peak indicator is selected.



Fix Size
When selected, the *Calibration Editor* box appears. Enter the correct basepair size of the peak and click **OK**.
NOTE: Only Sizes that occur in the selected Size Standard can be entered in the *Calibration Editor Size* field.
The peak will be “fixed” at the specified basepair position and all green triangle peak indicators to the left and right of the “fixed” peak will be adjusted to correctly align with the chosen Size Standard.



Peak 5086
☑ Fixed
Size 300.0

The *Fix Size* feature is useful when the selected Size Standard has uniformly spaced peaks and the sample ILS has additional peaks due to pull-up or other experimental abnormalities thereby influencing the pattern recognition algorithm.
NOTE: *Fix Size* is not active for manually added peaks or peaks outside the Size Standard range.

Reset Peaks
Select *Reset Peaks* to eliminate manually added peaks and/or extra green triangle peak indicators after *Fix Size*.
NOTE: Deleted peaks will not be recalled when *Reset Peaks* is selected.

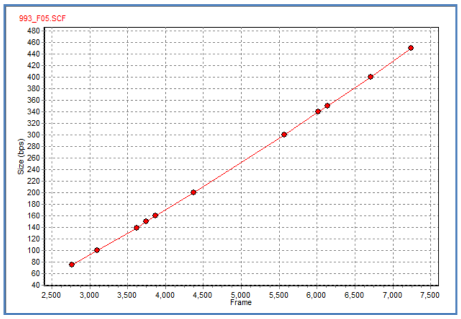
Update Calibration
After editing peaks in the *Sample ILS*, select *Update Calibration*. The *Match Score* for the sample will be recalculated based on the edited peak indicator positions. When *Size Calibration Charts* is closed, the *Size Match Score* indicators next to the filenames in the *Sample File Tree* in the *Main Analysis* window will be updated.

Copy Current Calibration Data
When selected, the frame position and basepair position of the green triangle peak indicators for the selected sample will be copied to the Windows clipboard and can be pasted into a spreadsheet or word processing program such as Microsoft Excel or Word.

Calibration Plots

The *Calibration Plots* chart the migration linearity of the ILS fragment peaks for each sample. The charts plot the peak basepair positions on the y-axis as a function of time (raw data frame numbers) on the x-axis. As the linearity of the line decreases so does the *Match Score* for the sample. Incorrectly identified peaks will result in a low *Match Score*.

Double-click a *Calibration Plot* to select the sample in the *Sample List* and display the sample in the *Sample ILS*. The currently selected sample filename will appear red in the upper left corner of the *Calibration Plot*.



Procedure

After a Size Standard has been chosen and the data is processed by the Run Wizard, the *Size Calibration Charts* can be used to correct improperly sized samples.

1. Click the **Size Calibration Charts** icon in the main toolbar

2. The *Calibration Charts* window appears
3. Select a sample to edit in the *Sample List*
4. The sample's ILS appears in the *Sample ILS* frame
5. Right-click in the *Sample ILS* frame and chose *Add, Delete*, or *Fix Size* to correct size call
6. Right-click again and select *Update Calibration*
7. The changes will be implemented for the sample and the *Match Score* will be updated
8. When editing is finished, close *Size Calibration Charts*
9. The *Size Match Score* indicators in the *Sample File Tree* of the *Main Analysis* window will be updated

Icons and Functions

Toolbar Icons

Size Calibration
Found in the main toolbar of the *Main Analysis* window.

View Mode
Change the layout of the *Calibration Plots* frame. Adjust the maximum number of rows and columns displayed. Maximum number of rows and columns is 5.

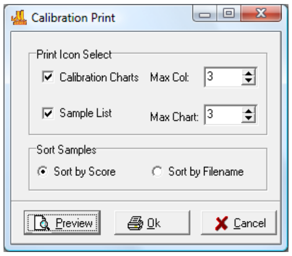
Chart Synchronize
When selected, both the *Expected Size Standard* and *Sample ILS* traces become synchronized. This option is not selected by default.

Preprocess Raw Data
Select *Preprocess Raw Data* to smooth the samples' raw data ILS.

Auto Fit Y
Provides the option to automatically fit the *Sample ILS's* y-axis by the maximum peak height in the trace OR by only the highest matched peaks.

Print
Launches Print Preview and formatting options to print the size calibration page(s).

Save
Options to save print calibration page(s).



Print Icon Select
☑ Calibration Charts Max Col: 3
☑ Sample List Max Chart: 3

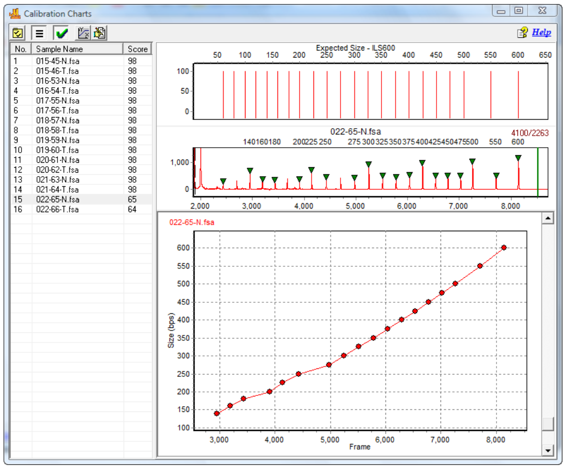
Sort Samples
● Sort by Score ○ Sort by Filename

Manual Calibration
Provides the option of manually entering standard peak sizes if many peaks have been modified. This window contains three columns: Standard Size (fragment sizes of standards used for size calling), Peak Position (in frames), and Size (sizes are automatically entered, but easily edited).

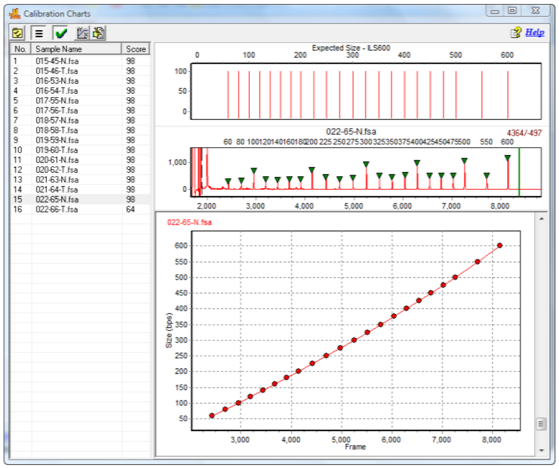
What to Expect

It is important to verify sizing accuracy prior to analyzing a dataset. If a sample is not sized correctly, peaks may be called Off Ladder (OL) if a panel is applied or will not be contained in the same Bin as other peaks in the case of AFLP analysis. Incorrect sizing most dramatically affects larger size fragments.

Before & After Editing Size Call


Calibration Charts
Help
Expected Size - ILS600
022-65-N.fsa
4100/2263
Size (bps)
Frame

No.	Sample Name	Score
1	015-45-N.fsa	98
2	015-46-T.fsa	98
3	016-53-N.fsa	98
4	016-54-T.fsa	98
5	017-55-N.fsa	98
6	017-56-T.fsa	98
7	018-57-N.fsa	98
8	018-58-T.fsa	98
9	019-59-N.fsa	98
10	019-60-T.fsa	98
11	020-61-N.fsa	98
12	020-62-T.fsa	98
13	021-63-N.fsa	98
14	021-64-T.fsa	98
15	022-65-N.fsa	65
16	022-66-T.fsa	64


Calibration Charts
Help
Expected Size - ILS600
022-65-N.fsa
4364/-497
Size (bps)
Frame

No.	Sample Name	Score
1	015-45-N.fsa	98
2	015-46-T.fsa	98
3	016-53-N.fsa	98
4	016-54-T.fsa	98
5	017-55-N.fsa	98
6	017-56-T.fsa	98
7	018-57-N.fsa	98
8	018-58-T.fsa	98
9	019-59-N.fsa	98
10	019-60-T.fsa	98
11	020-61-N.fsa	98
12	020-62-T.fsa	98
13	021-63-N.fsa	98
14	021-64-T.fsa	98
15	022-65-N.fsa	98
16	022-66-T.fsa	64

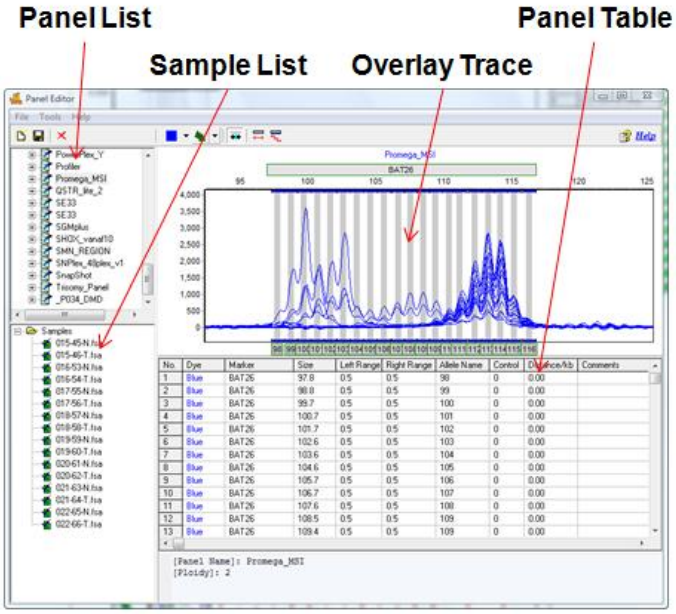
Chapter 5 Panel Editor

Chapter 5 Panel Editor
Overview
Procedure
Icons and Functions
What to Expect

Overview

The *Panel Editor* can be accessed from the *Tools* menu in the *Main Analysis* window *OR* via the **Panel Editor** icon in the Run Wizard *Template Selection* box. The purpose of a Panel is to outline the position of expected alleles. Loci or Markers give a range where a group of alleles is expected to appear and Bins indicate the specific basepair position of the expected allele. In GeneMarker, Markers are indicated by a horizontal gray bar across the top of the electropherogram. Bins are indicated by the dye-colored brackets at the top and bottom of the electropherogram. Only in the *Panel Editor* do the vertical gray bars within the electropherogram indicate the center of the Bin. For all other views in GeneMarker, the vertical gray bars in the electropherogram indicate the center of the detected peak.

Panel Editor



No.	Dye	Marker	Size	Left Range	Right Range	Allele Name	Control	Distance/kb	Comments
1	Blue	BAT26	97.8	0.5	0.5	98	0	0.00	
2	Blue	BAT26	98.8	0.5	0.5	99	0	0.00	
3	Blue	BAT26	99.7	0.5	0.5	100	0	0.00	
4	Blue	BAT26	100.7	0.5	0.5	101	0	0.00	
5	Blue	BAT26	101.7	0.5	0.5	102	0	0.00	
6	Blue	BAT26	102.6	0.5	0.5	103	0	0.00	
7	Blue	BAT26	103.6	0.5	0.5	104	0	0.00	
8	Blue	BAT26	104.6	0.5	0.5	105	0	0.00	
9	Blue	BAT26	105.7	0.5	0.5	106	0	0.00	
10	Blue	BAT26	106.7	0.5	0.5	107	0	0.00	
11	Blue	BAT26	107.6	0.5	0.5	108	0	0.00	
12	Blue	BAT26	108.5	0.5	0.5	109	0	0.00	
13	Blue	BAT26	109.4	0.5	0.5	109	0	0.00	

```
[Panel Name]: Promega_MSI
[Ploidy]: 2
```

Panel List

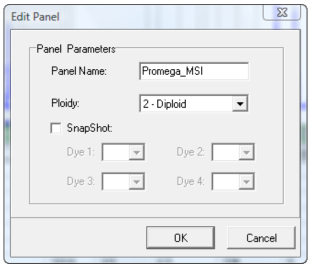
The *Panel List* includes a list of all pre-defined and custom Panels saved to the Panels folder in the SoftGenetics GeneMarker directory. Single left-click on the Panel name to display the Panel in the *Overlay Trace* frame. Double-click the Panel name to expand the folder and view the Markers associated with the Panel. Single left-click the Marker name to display that Marker in the *Overlay Trace* frame.

Additional Options

To see additional options for each Panel, right-click the Panel name and the right-click menu will appear with the following options.

Edit Panel

Opens the *Edit Panel* box. Editing the *Panel Name* field will change how the Panel is labeled in *Panel Editor*. Set the *Ploidy* from Monoploid (1) to Decaploid (10). If the number of peaks within a Marker exceeds the *Ploidy* setting, the additional peaks will be labeled Off Ladder (OL) and given the Undetermined (red) Quality rank and PL Quality Reasoning. **See Chapter 7 Special Applications – SNaPshot** regarding the *SNaPshot* feature in *Edit Panel*.



Panel Name: Promega_MSI

Ploidy: 2 - Diploid

☐ SnapShot

Delete Panel

Select *Delete* (**Hot Key** = **DEL**) to delete the Panel from the *Panel List* and from the SoftGenetics GeneMarker directory.

NOTE: This action is irreversible.

Export Panel

Opens the *Save As* window. Choose a directory folder and click **Save**. The Panel will be copied to the selected directory and will also remain in the *Panel List* and SoftGenetics GeneMarker Panel directory. The Panel will be exported as an XML file which can be opened with Internet Explorer, Microsoft Excel, or Notepad.

Reload Panel

Click *Reload* to undo editing changes to the Panel. The most recently saved Panel will be restored.
NOTE: If the user selects *Save Panel* and then answers "NO" to the "The Panel has been changed, save changes to file?" the changes will remain in the *Overlay View* until the user chooses *Reload Panel* or GeneMarker program is closed.

Sample List

The *Sample List* contains all the samples uploaded to GeneMarker in the current project. Samples with a checkmark next to the filename will be displayed in the *Overlay View*. Double-click the sample filename OR right-click the sample and choose *Select/De-Select* to enable/disable it in the *Overlay View*. Right-click any sample in the list and choose *Select All/De-Select All* to display all or no sample traces in the *Overlay View*.

Sorting Options

Sample Name

Sorts the samples in alphanumeric descending order. *Sample Name* sorting is the default option.

Size Score

Sorts the samples by the lane size score as it appears in the *Size Calibration Charts* (**See Chapter 4 Fragment Sizing Standards**). Samples with higher scores will appear at the top of the list.

Overlay Trace

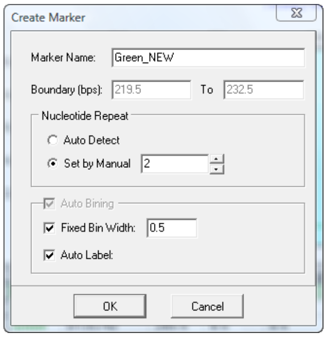
The *Overlay Trace* displays all selected samples in the *Sample List*. The Marker bars appear above the electropherogram and the Bins appear within the electropherogram as brackets at the top and bottom. The center of the Bin is indicated by the vertical grey bar in the electropherogram (only in *Panel Editor*). The *Overlay Trace* view can be changed by clicking the **Trace Mode** icon in the toolbar. Other options include *Max & Average* and *Gel Image*.

Navigation in the *Overlay Trace* frame is similar to the navigation options in the *Main Analysis* window. **See Chapter 3 Main Analysis Overview.**

Marker Options

Create Marker

Hold down **CTRL** key and left-click and drag across peaks in the *Overlay View*. A light-blue hashed box will appear. Right-click in the hashed box and select *Create Marker*. The *Create Marker* box appears. Adjust parameters and click **OK**.



Marker Name: Green_NEW
Boundary (bps): 219.5 To 232.5
Nucleotide Repeat
○ Auto Detect
● Set by Manual 2
☑ Auto Bining
☑ Fixed Bin Width: 0.5
☑ Auto Label:

Marker Name: Edit the *Marker Name* field to change how the Marker will be labeled in the Panel.
Boundary: The basepair range of the Marker is defined by the range of the light-blue hashed box and is therefore inactive in the *Create Marker* box. To edit the *Boundary*, see *Edit Marker* below.

Nucleotide Repeat

Auto Detect: Based on the peaks present in the *Overlay View*, GeneMarker will attempt to detect the number of nucleotides in each repeat unit of the alleles and place Bins at the appropriate interval.
Set by Manual: Select this option if the number of nucleotides in the allele repeat unit is known and GeneMarker will place Bins at the specified interval.

Auto Binning

Fixed Bin Width: Check this option to enter the number of basepairs on the right and left of the center of the Bins. If 0.5 is selected as the Bin Width then the total Bin range will be 1.0 basepairs.
Auto Label: When deselected, the Bins are automatically labeled with the basepair size of the Bin position to the nearest tenth of a basepair. If selected, the basepair size is rounded up to a whole number value.

Edit Marker

Double-click the Marker bar *OR* right-click the Marker bar and select *Edit Marker*. The *Edit Marker* box appears. Adjust parameters and click **OK**.

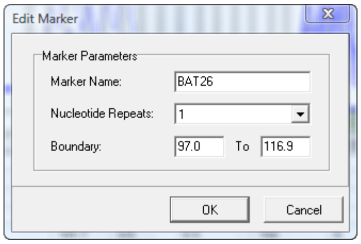
NOTE: The *Edit Marker* box can also be accessed by right-clicking the Marker name in the *Panel List* and selecting *Edit*.

Marker Parameters

Marker Name: Edit the *Marker Name* field to change how the Marker will be labeled in the Panel.

Nucleotide Repeats: Use the *Nucleotide Repeats* drop-down menu (1-6) or enter a value into the field to set the number of basepairs expected between each allele in the Marker.

Boundary: To move a Marker left or right, hold down **SHIFT** key and left-click and drag the Marker bar. To adjust the basepair range over which a Marker is located, hold down **SHIFT** key and mouse-over the edge of the Marker bar until a double-headed arrow appears then left-click and drag the Marker edge to increase or decrease the range *OR* right-click the Marker bar and select *Edit Marker*. The *Edit Marker* box appears. Adjust the *Boundary* field values as necessary.



Marker Name: BAT26
Nucleotide Repeats: 1
Boundary: 97.0 To 116.9

Additionally, if a Marker needs only slight adjustment to the right or left, right-click the Marker bar and select *Adjust Marker*. The Marker will move automatically to align with the closest peaks.

Edit Marker Bins

Right-click the Marker bar and select *Update Alleles*. The *Update Marker Alleles* box will appear. Adjust parameters and click **OK**.

Nucleotide Repeat

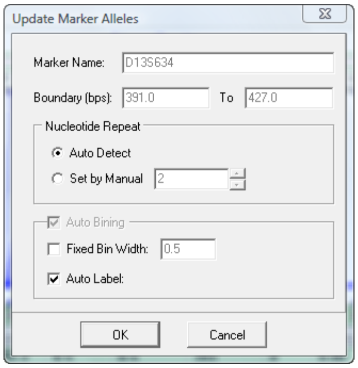
Auto Detect: Based on the peaks present in the *Overlay View*, GeneMarker will attempt to detect the number of nucleotides in each repeat unit of the alleles and place Bins at the appropriate interval.

Set by Manual: Select this option if the number of nucleotides in the allele repeat unit is known and GeneMarker will place Bins at the specified interval.

Auto Binning

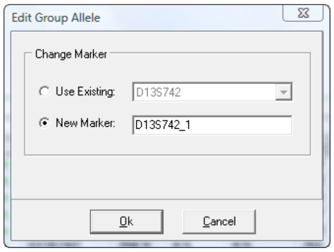
Fixed Bin Width: Check this option to enter the number of basepairs on the right and left of the center of the Bins. If 0.5 is selected as the Bin Width then the total Bin range will be 1.0 basepairs.

Auto Label: When deselected, the Bins will be automatically labeled with the basepair size of the Bin position to the nearest tenth of a basepair. If selected, the number will be rounded up to a whole number value.



Marker Name: D13S634
Boundary (bps): 391.0 To 427.0
● Auto Detect
○ Set by Manual 2
☑ Auto Bining
☐ Fixed Bin Width: 0.5
☑ Auto Label

To associate Bins with a different Marker, hold down **CTRL** key and left-click and drag across peaks at the edge of a Marker. A light blue hashed box will appear. Right-click in the hashed box and select *Change Marker*. The *Edit Group Allele* box will appear. Select *New Marker* and a pre-defined name will appear. Use this Marker label or create a new name and click **OK**. The highlighted Bins are now incorporated into the newly created Marker.



○ Use Existing: D13S742
● New Marker: D13S742_1

Delete Marker

Right-click the Marker bar and select *Delete Marker* *OR* right-click the Marker name in the *Panel List* and select *Delete* (**Hot Key** = **DEL**).

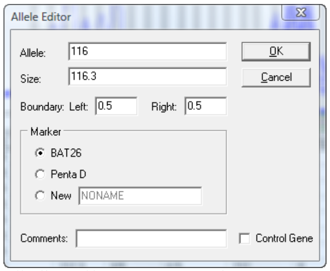
Bin Options

Create Bin

To create a Bin position, right-click in the electropherogram at the exact position to place the new Bin. Select *Insert Allele*. The *Allele Editor* box will appear. Adjust parameters and click **OK**.

Allele: Enter a name for the Bin. All peaks that appear within the Bin will display this value in the Allele Label in the *Main Analysis* window.

Size: Indicates the basepair position of the center of the Bin.



Allele: 116
Size: 116.3
Boundary: Left: 0.5 Right: 0.5
Marker
● BAT26
○ Penta D
○ New NONAME
Comments:
☐ Control Gene

Boundary: Indicates the range of the Bin on either side (*Left* and *Right*) of the Bin center.

Marker: Select which Marker to associate the Bin with. The Markers to the right and left of the Bin position will be displayed as well as the option to create a new Marker for the Bin. All Bins must be associated with a Marker.

Comments: Free-form text field to associate a comment with the Bin.

Control Gene: Select *Control Gene* if the peaks that fall within the Bin are of consistent height. Peaks that appear within the *Control Gene* designated Bins will be used to normalize the data. If a Bin is marked as a *Control Gene*, a "1" will appear in the *Control* column of the *Panel Table*. Additionally, right-click the center Bin vertical grey bar in the *Overlay Trace* and select *Set as Control/Non-Control* to enable/disable *Control Gene*.

NOTE: *Control Gene* is for use with MLPA analysis. **See Chapter 7 Special Applications – MLPA**.

Edit Bin

Right-click the vertical grey bar in the center of the Bin in the *Overlay Trace*. Select *Edit Allele* and the *Allele Editor* box appears. Adjust parameters and click **OK**. See *Create Bin* section above for explanation of *Allele Editor* options.

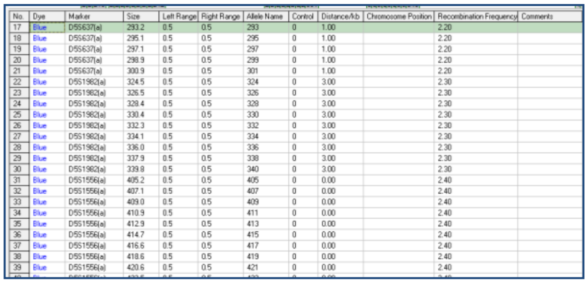
To move a bin, hold down **SHIFT** key and left-click and drag the vertical grey bar in the center of the Bin to the right or left. Let go of the **SHIFT** key and mouse button and the Bin will remain in place. To edit the range of a Bin in the *Overlay View*, click the **Trace Mode** icon to view the *Gel Image*. Hold down **SHIFT** and mouse over the vertical white line of the Bin edge. When a double-headed arrow appears, hold down left-click and drag the Bin edge to adjust the range.

Delete Bin

Right-click the vertical grey bar in the center of the Bin in the *Overlay Trace*. Select *Delete Allele*. The Bin will be deleted from the Panel. To delete multiple Bins, hold down **CTRL** key and left-click and drag across peaks in the *Overlay View*. A light-blue hashed box will appear. Right-click in the hashed box and select *Delete Alleles*. The Bins highlighted by the hashed box will be removed from the Panel.

Panel Table

The *Panel Table* displays Marker and Bin information for the dye color displayed in the *Overlay Trace* frame. All columns except *Dye* and *Marker* can be edited in the *Panel Table*. Right-click a highlighted cell and select *Set Value to Column* to make all values in the column equal to the value in the highlighted cell.



No.	Dye	Marker	Size	Left Range	Right Range	Allele Name	Control	Distance/kb	Chromosome Position	Recombination Frequency	Comments
17	Blue	D5S637(a)	293.2	0.5	0.5	293	0	1.00		2.20	
18	Blue	D5S637(a)	295.1	0.5	0.5	295	0	1.00		2.20	
19	Blue	D5S637(a)	297.1	0.5	0.5	297	0	1.00		2.20	
20	Blue	D5S637(a)	298.9	0.5	0.5	299	0	1.00		2.20	
21	Blue	D5S637(a)	300.9	0.5	0.5	301	0	1.00		2.20	
22	Blue	D5S1982(a)	324.5	0.5	0.5	324	0	3.00		2.30	
23	Blue	D5S1982(a)	326.5	0.5	0.5	326	0	3.00		2.30	
24	Blue	D5S1982(a)	328.4	0.5	0.5	328	0	3.00		2.30	
25	Blue	D5S1982(a)	330.4	0.5	0.5	330	0	3.00		2.30	
26	Blue	D5S1982(a)	332.3	0.5	0.5	332	0	3.00		2.30	
27	Blue	D5S1982(a)	334.1	0.5	0.5	334	0	3.00		2.30	
28	Blue	D5S1982(a)	336.0	0.5	0.5	336	0	3.00		2.30	
29	Blue	D5S1982(a)	337.9	0.5	0.5	338	0	3.00		2.30	
30	Blue	D5S1982(a)	339.8	0.5	0.5	340	0	3.00		2.30	
31	Blue	D5S1556(a)	405.2	0.5	0.5	405	0	0.00		2.40	
32	Blue	D5S1556(a)	407.1	0.5	0.5	407	0	0.00		2.40	
33	Blue	D5S1556(a)	409.0	0.5	0.5	409	0	0.00		2.40	
34	Blue	D5S1556(a)	410.9	0.5	0.5	411	0	0.00		2.40	
35	Blue	D5S1556(a)	412.9	0.5	0.5	413	0	0.00		2.40	
36	Blue	D5S1556(a)	414.7	0.5	0.5	415	0	0.00		2.40	
37	Blue	D5S1556(a)	416.6	0.5	0.5	417	0	0.00		2.40	
38	Blue	D5S1556(a)	418.6	0.5	0.5	419	0	0.00		2.40	
39	Blue	D5S1556(a)	420.6	0.5	0.5	421	0	0.00		2.40	

Dye

Indicates the dye color of the Bin.

Marker

Indicates which Marker the Bin is contained in.

Size

Indicates the position of the Bin center in basepairs.

Left/Right Range

Indicates the range of the Bin on either side of the Bin center.

Allele Name

Peaks that appear within the Left/Right Range of the Bin will be labeled with the Allele Name.

Control

Bins marked with a "1" are considered Control Bins. Enter "0" in Control column of any allele that the contrasting color is needed (example: Abbott Cystic Fibrosis mutant alleles, non-controls) in the electropherogram of the Main Analysis Screen. Bins marked with a "0" are not controls and will not be used for data normalization in MLPA Analysis. Bins marked with a -1 (negative 1) are displayed as quality control fragments for MLPA analysis final report. **See Chapter 7 Special Applications – MLPA**.

Distance/kb
Allows the user to input the distance (in kb) that each allele is from the beginning of the sequence. For example, "38.1" means that the allele is 38.1 kb from the beginning of the sequence for MLPA Analysis, or in the case of Haplotype Analysis the Mb distance is used to order the markers in the pedigree diagram. A 0 is used to indicate the marker to be located at the middle of the marker list, positive numbers below the 0 marker and negative numbers are above the 0 marker in Haplotype Analysis.

NOTE: Applicable for **MLPA Analysis**. **See Chapter 7 Special Applications – MLPA**.
NOTE: Application for Haplotype analysis. **See Chapter 7 Special Applications – Haplotype Analysis**

Recombination Frequency – Used in Haplotype analysis pedigree diagram to report the Genetic distance between markers

Comments
Free-form text field to associate a comment with the Bin.

Procedure

As mentioned previously, Panels are created to outline the position, in basepairs, of expected peaks. In GeneMarker, the Panels associated with several commercially distributed genotyping kits are included. Examples of some of the pre-defined Panels include MRC Holland's MLPA kits, Promega's MSI kit, and ABI's forensic human identity kits. GeneMarker also offers the opportunity to create a new custom Panel if the pre-defined Panels do not include a kit that the user is working with. Below is a discussion of how to use the pre-defined Panels or create a new Panel with GeneMarker's *Panel Editor* tool.

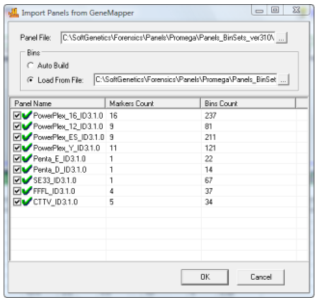
The Panels displayed by default include:

MLPA_CMT1_P033_REX
MLPA_DMD_P034_REX
MLPA_DMD_P035_REX
MLPA_MSH2_MLH1_P003_REX
MLPA_VHL_P016_REX
HID_IDENTIFILER
HID_POWERPLEX_16
HID_SGMPLUS
PROMEGA_MSI
SNPLEX_48PLEX_1

Pre-Defined Panels

In addition to the Panels displayed by default, the user has the option to import standard Panels and Bins Text files and to access additional commercial kit panels including: MRC Holland MLPA Panels, Trisomy Panels for Aneufast, Devyeser, Finnzymes and GenProbe kits, Cystic Fibrosis Panels for Abbott and GenProbe kits.

Import ABI Panels and Bins Files



Panel File: C:\SoftGenetics\Forensics\Panels\Promega\Panels_BinSets_ver310\
Bins
○ Auto Build
● Load From File: C:\SoftGenetics\Forensics\Panels\Promega\Panels_BinSet

Panel Name	Markers Count	Bins Count
☑ ✓ PowerPlex_16_ID3.1.0	16	237
☑ ✓ PowerPlex_12_ID3.1.0	9	81
☑ ✓ PowerPlex_ES_ID3.1.0	9	211
☑ ✓ PowerPlex_Y_ID3.1.0	11	121
☑ ✓ Penta_E_ID3.1.0	1	22
☑ ✓ Penta_D_ID3.1.0	1	14
☑ ✓ SE33_ID3.1.0	1	67
☑ ✓ FFFL_ID3.1.0	4	37
☑ ✓ CTTV_ID3.1.0	5	34

1. In *Panel Editor*, select *File → Import ABI Panels* from the menu bar.
2. The *Import Panels from GeneMapper* box appears.
3. Click the access button next to the *Panel File* field. A Windows Explorer window will appear.
4. Navigate to the location of the Panels.txt file and click **Open**.
5. Next, click the access button next to the *Bins Load from File* field and locate the Bins.txt file.
6. Click **Open**.

NOTE: Select *Bins Auto Build* if a Bins.txt file does not exist.

7. Click **OK** in the *Import Panels from GeneMapper* box.
8. All Panels in the Panels.txt file will be uploaded into GeneMarker.
9. Select a newly uploaded Panel from the *Panel List*.
10. Edit the Markers and Bins so that they align with the peaks in the dataset.
11. Save the edited Panel and close *Panel Editor*.
12. Click the **Run Project** icon in the *Main Analysis* window.
13. Select the Panel from the *Panel* field in the Run Wizard *Template Selection* box.
14. Proceed through Run Wizard and data analysis. **See Chapter 2 General Procedure and Chapter 3 Main Analysis Overview.**

MLPA Panels

1. In *Panel Editor*, select *File → Import Pre-defined Panels* from the menu bar.
2. The SoftGenetics GeneMarker Pre-defined Panels folder will appear.
3. Open the MLPA Folder
4. Choose a MRC Holland MLPA Panel for upload.

NOTE: Go to www.mrc-holland.com/products.htm for additional information on MLPA Panels.

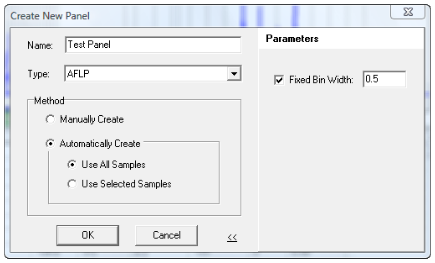
5. Click **Open**.
6. The MLPA Panel will appear in the *Panel List*.
7. Follow steps **9-14** above.

Custom Panel Creation

Follow the steps below to create a new Panel based on the dataset currently uploaded to GeneMarker.

Automatic Panel Creation

1. In *Panel Editor*, select *File → Create New Panel* from the menu bar or click the **Create New Panel** icon.
2. The *Create New Panel* box appears.
3. Enter a name for the Panel in the *Name* field. This will be the Panel name that is displayed in the *Panel List*.
4. The *Type* will, by default, display the *Analysis Type* chosen initially in Run Wizard *Template Selection*. Choose a different *Type* from the drop-down list if required to match the *Analysis Type* selected.
    a. *Fragment (Animal), Fragment (Plant),* and *HID* Types several Markers per dye color will be created based on peak grouping in the dataset
    b. *AFLP, MLPA,* and *SNPlex* will create just one Marker for each dye color
    c. *SNaPshot* Type will associate nucleotides (ATCG) with each dye color
5. Select *Automatically Create*
    a. *Use All Samples* will create a Panel based on an overlay of all the sample peaks in the dataset
    b. *Use Selected Samples* will create a Panel based only on the samples selected in the *Panel Editor Sample List*


Create New Panel
Parameters
Method
○ Manually Create
Use All Samples
○ Use Selected Samples
OK
Cancel
<<

Name: Test Panel
Type: AFLP
● Automatically Create
● Use All Samples
☑ Fixed Bin Width: 0.5

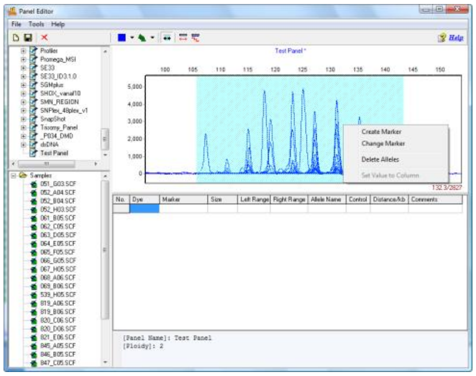
6. Click the double-arrow button to expand the dialog box and see additional parameters
7. If required, check the *Fixed Bin Width* option and enter a value for the left and right Bin ranges
8. When finished, click **OK**
9. The new Panel will be created and added to the **Panel List**

NOTE: New Panels are created based on the *Max & Average View Mode*. More intense peaks are given higher priority for Bin placement when peaks do not overlap perfectly.

10. Edit the Markers and Bins as described in the previous section – *Panel Editor Overview*.
11. Follow steps **9-14** above.

Manual Panel Creation

1. In *Panel Editor*, select *File → Create New Panel* from the menu bar or click the **Create New Panel** icon.
2. Enter a Panel name in the *Name* field
3. Choose the appropriate *Analysis Type* from the *Type* drop-down menu
4. Select *Manually Create*
5. When finished, click **OK**
6. The Panel name will appear in the *Panel List*; however, no Markers or Bins will be associated with the Panel
7. Follow the steps in the previous section – *Panel Editor Overview* – to create Markers and Bins


Panel Editor
File
Tools
Help
Help
Profiler
Promega_MSI
SE33
SE33_ID3.1.0
SGMplus
SHOX_vanal10
SMN_REGION
SNPlex_48plex_v1
SnapShot
Trisomy_Panel
_P034_DMD
dsDNA
Test Panel
Samples
051_G03.SCF
052_A04.SCF
052_B04.SCF
052_H03.SCF
061_B05.SCF
062_C05.SCF
063_D05.SCF
064_E05.SCF
065_F05.SCF
066_G05.SCF
067_H05.SCF
068_A06.SCF
069_B06.SCF
539_H05.SCF
819_A06.SCF
819_B06.SCF
820_C06.SCF
820_D06.SCF
821_E06.SCF
845_A05.SCF
846_B05.SCF
847_C05.SCF
Test Panel *
100
105
110
115
120
125
130
135
140
145
150
5,000
4,000
3,000
2,000
1,000
0
Create Marker
Change Marker
Delete Alleles
Set Value to Column
132.3/2827
No.
Dye
Marker
Size
Left Range
Right Range
Allele Name
Control
Distance/kb
Comments

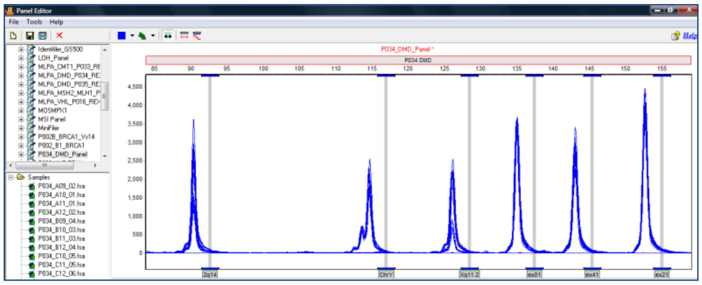
```
[Panel Name]: Test Panel
[Ploidy]: 2
```

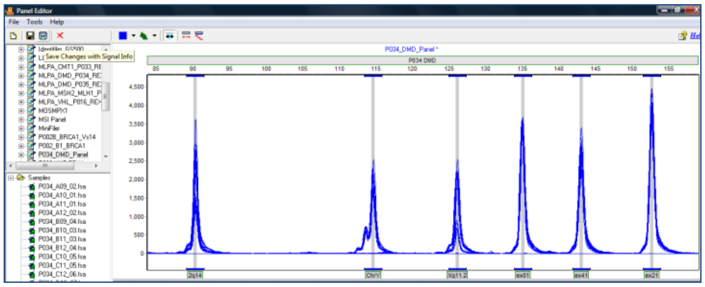
Adjusting and Calibrating Panels

It is common for panel alignment to be shifted due to variations in genetic analyzers or run conditions (such as templerature, injection time). Markers or bins can be manually aligned to the allele ladder using the shift and mouse key. Once a panel has been adjusted to fit the output of a specific genetic analyzer, the panel should be saved with the signal information. The combination of the panel bins and signal information provides GeneMarker's pattern recognition algorithm with the data necessary to use major and minor auto panel adjust icons to align the panel for projects from future runs on that same analyzer. If more than one genetic analyzer is used, a separate panel for each genetic analyzer should be saved with signal information, for example: panelname_ABI3100, panelname_ABI3130, panelname_CEQ8000.

Align all of the bins within a marker

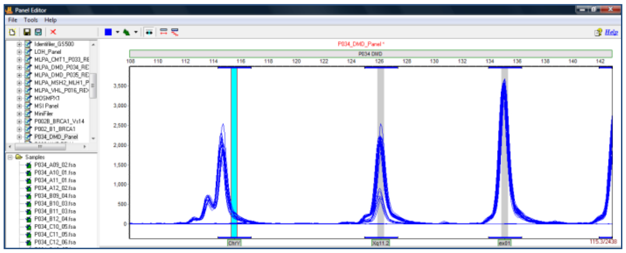
1. Hold down the shift key
2. At the same time place the mouse over the gray marker name bar at the top of the electropherogram
3. The marker rectangle will be outlined in red and the panel name will be in red font when the adjust feature is active
4. Drag the marker to align the bins with the peaks of the allelic ladder
5. Save the panel with signal information (the **turquoise save icon**) to enable the major panel adjust feature to work in future projects

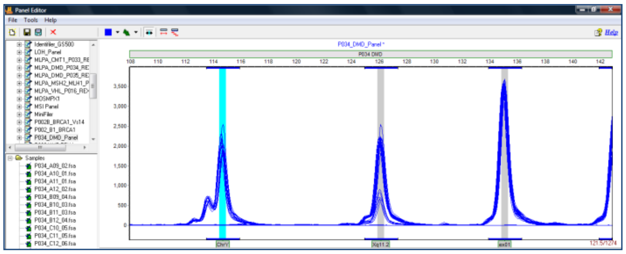

Panel Editor
File Tools Help
P034_DMD_Panel*
P034 DMD
Samples

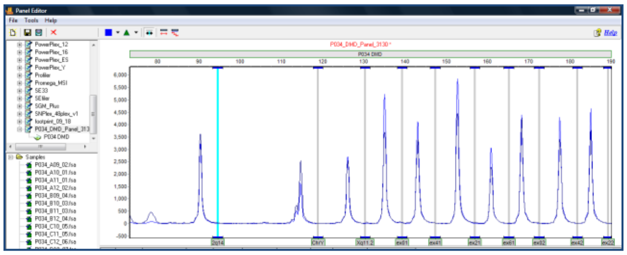

Panel Editor
File Tools Help
Save Changes with Signal Info
P034_DMD_Panel*
P034 DMD
Samples

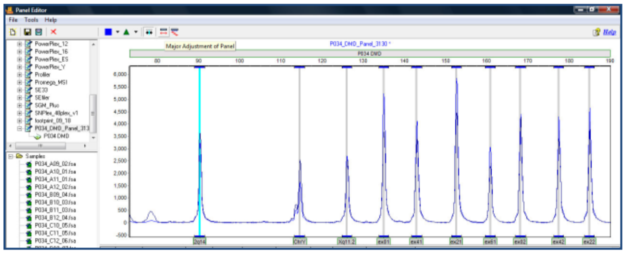
Align an individual bin

1. Select the gray vertical bar of the bin with the mouse – the bar will turn blue
2. Hold down the shift key and click on the gray, vertical bar for the bin
3. The vertical bar will be outlined in red and the panel name will be in red font
4. Use the mouse to drag the gray vertical bar to the center of the peak


Panel Editor
File Tools Help
P034_DMD_Panel*
P034 DMD


Panel Editor
File Tools Help
P034_DMD_Panel*
P034 DMD





Major and minor Auto-panel adjust

1. Major panel adjustment icon should be used with a panel that was previously saved with signal information from the genetic analyzer if the data run is shifted 1-5 base pairs from alignment.
2. Click on the auto-major panel adjustment icon
3. The panel is automatically adjusted for this project
4. Minor panel adjustment icon aligns the center of the Bin to the center of the nearest peak (within one basepair of the Bin).

Icons and Functions

The following are explanations of menu and icon options in *Panel Editor*.

Menu Options

The *Panel Editor* contains three menu options – *File, Tools*, and *Help*. The *File* menu allows the user to create, save, and export Panels. The *Tools* menu contains options for datasets with allelic ladder samples and exporting a Panel. The *Help* menu contains navigation hints for *Panel Editor*.

File Menu

Create New Panel

Launches the *Create New Panel* dialog box with the options to create a new Panel *Automatically* or *Manually*

Delete Current Panel/Marker

Deletes the Panel or Marker that is currently highlighted in the *Panel List*

Save Changes

Saves edits and changes to the Panel in the SoftGenetics GeneMarker Panel directory (**Hot Key** **= CTRL+S**)

Save as New Panel

Opens the *Input Dialog* box with a field to enter a new Panel name. The Panel is added to the *Panel List* and saved in the SoftGenetics GeneMarker Panel directory.

Import Panels

Opens a Windows Explorer window to the same folder the sample files were uploaded from. Use the *Import Panels* option to find previously exported Panel Files (.xml) on local or networked computers.

Import Pre-Defined Panels

Opens the SoftGenetics GeneMarker PreDefined Panels Folder. This folder contains many panels from commercially available chemistries: Aneuploidy/Trisomy (to import panels for Aneufast, Devyser, Elucigene, and Finnzymes)Cystic Fibrosis (to import panels from Abbott and Elucigene) and the MLPA Panel folder (to import additional MRC Holland MLPA Panels).

Import ABI Panels

Launches the *Import Panels from GeneMapper* box. Opens Panels and Bins Text files and converts them to single Panel files in XML format for use in GeneMarker.

Export Panel

Exports the currently selected Panel in the *Panel List* as an XML file to a specified directory on a local or network computer.

Exit

Closes the *Panel Editor* tool. Be sure to save changes to the Panel before exiting.

Tools Menu

Match Ladder

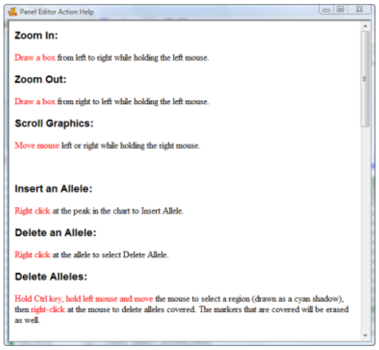
Opens the *Select Ladder* box. Choose an allelic ladder sample from the drop-down menu. Click **OK** and the Panel will adjust slightly to align with the peaks in the selected ladder sample.
NOTE: Large differences between peak and Bin position cannot be resolved with the *Match Ladder* function.

Export the Project Panel

Exports the currently selected Panel in the *Panel List* as an XML file to a specified directory on a local or network computer.

Help Menu

The *Help* menu contains a link to *Hot Keys* in *Panel Editor*. Click *Hot Keys* and the *Panel Editor Action Help* box appears.



Zoom In:

Draw a box from left to right while holding the left mouse.

Zoom Out:

Draw a box from right to left while holding the left mouse.

Scroll Graphics:

Move mouse left or right while holding the right mouse.

Insert an Allele:

Right click at the peak in the chart to Insert Allele.

Delete an Allele:

Right click at the allele to select Delete Allele.

Delete Alleles:

Hold Ctrl key, hold left mouse and move the mouse to select a region (drawn as a cyan shadow), then right-click at the mouse to delete alleles covered. The markers that are covered will be erased as well.

Toolbar Icons

Panel Editor
Found in the Run Wizard *Template Selection* box OR the *Tools* menu.

Create New Panel
Opens the *Create New Panel* box. Follow the steps in the *Create a Custom Panel* section above.

Save Changes
Permanently saves Panel edits to the currently opened Panel file which is located in the SoftGenetics GeneMarker Panel directory.

Save Changes with Signal Info
Permanently saves all Panel edits, including height information which is used with the *Major Panel Adjustment* feature.
NOTE: A Panel must be correctly aligned with peaks in the dataset before selecting *Save Changes with Signal Info* in order for the *Major Panel Adjustment* feature to work correctly.

Delete Current Panel/Marker
Deletes whichever Panel or Marker is currently highlighted in the *Panel List*. This action is irreversible.

Show Dye
Allows the user to select a single dye color to view in the *Overlay View*. Cycle through the colors by left-clicking on the icon.

Trace Mode

Single left-click to cycle through the options or use the drop-down menu.

Trace Overlay displays all traces of the selected samples in the *Samples List* one dye color at a time. Single click any trace in the *Trace Overlay* frame and the trace will become bold and the associated sample will be highlighted in the *Sample List*.

Max & Average displays two traces in the electropherogram. The darker color line corresponds to the maximum peak height at that position and the lighter color line corresponds to the average of all selected sample traces at that position.

Gel Image displays selected samples as a synthetic gel image. Bin ranges in the *Gel Image* mode appear as white vertical lines and can be manipulated by holding down **SHIFT** and dragging the white lines left or right.

Check Range in Edit
When activated, the software will warn the user if they set the left or right range of an allele to overlap with another allele. This feature will prevent the user from setting allele boundaries too close to neighboring alleles. This option is selected by default.

Major Adjustment of Panel
Uses previously defined size and height information located in the Panel file to identify Marker and Bin positions. To be used when a Panel must be adjusted by 1 - 5 basepairs in order to align with the dataset peaks.
NOTE: A Panel must be correctly aligned with peaks in the dataset before selecting *Save Changes with Signal Info* in order for the *Major Panel Adjustment* feature to work correctly.

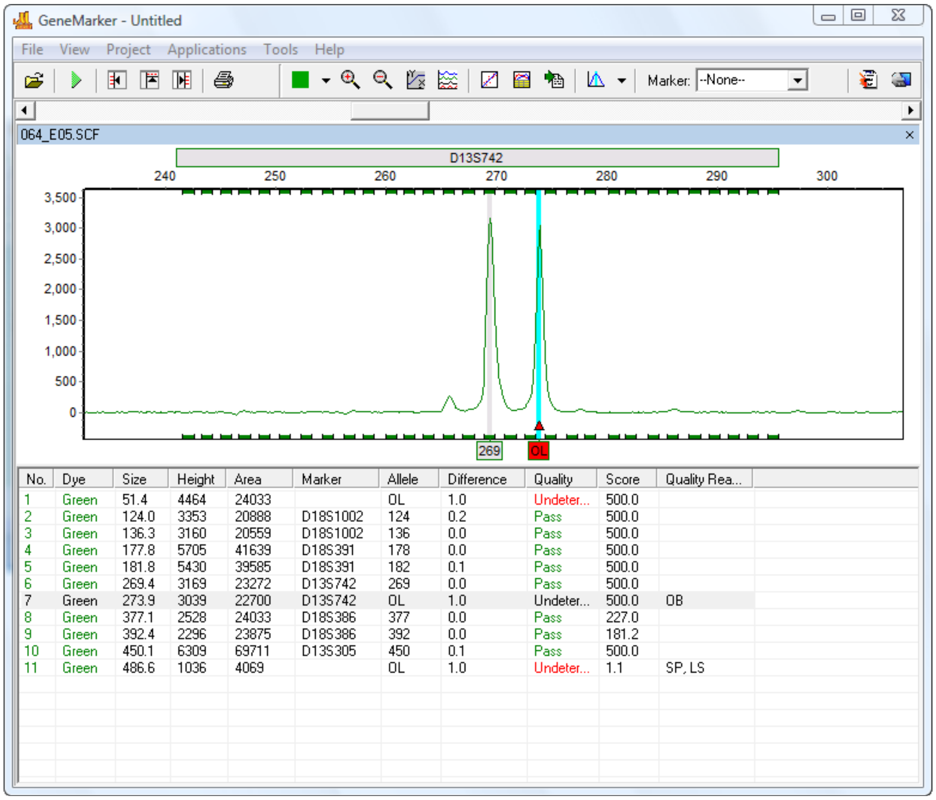
Minor Adjustment of Panel
Aligns the center of the Bin to the center of the nearest peak (within one basepair of the Bin).

What to Expect

Once a Panel has been created, it can be applied to the dataset. Save the edited Panel in *Panel Editor* then exit the *Panel Editor*. If the *Panel Editor* was accessed via the Run Wizard *Template Selection* box icon, then the selected Panel will appear in the *Panel* field. If the *Panel Editor* was accessed via the *Tools* menu then, Click the **Run Process** icon in the *Main Analysis* toolbar. The Run Wizard will appear. Select the Panel from the *Panel* drop-down menu in the Run Wizard *Template Selection* box. Proceed through the other Run Wizard boxes and click **OK** when the *Data Process* window is complete. The Panel will be applied.

After the Panel is applied to the dataset, the Markers and Bins appear in the *Electropherogram* and *Report Table*. In the *Electropherogram*, the Markers are horizontal grey bars, the Bins appear as dye-colored brackets above and below the trace, and the center of the peaks are marked with a vertical grey bar. Peaks that fall outside of the Markers or Bins of the Panel are marked Off Ladder (OL).

Off Ladder Allele



No.	Dye	Size	Height	Area	Marker	Allele	Difference	Quality	Score	Quality Rea...
1	Green	51.4	4464	24033		OL	1.0	Undeter...	500.0	
2	Green	124.0	3353	20888	D18S1002	124	0.2	Pass	500.0	
3	Green	136.3	3160	20559	D18S1002	136	0.0	Pass	500.0	
4	Green	177.8	5705	41639	D18S391	178	0.0	Pass	500.0	
5	Green	181.8	5430	39585	D18S391	182	0.1	Pass	500.0	
6	Green	269.4	3169	23272	D13S742	269	0.0	Pass	500.0	
7	Green	273.9	3039	22700	D13S742	OL	1.0	Undeter...	500.0	OB
8	Green	377.1	2528	24033	D18S386	377	0.0	Pass	227.0	
9	Green	392.4	2296	23875	D18S386	392	0.0	Pass	181.2	
10	Green	450.1	6309	69711	D13S305	450	0.1	Pass	500.0	
11	Green	486.6	1036	4069		OL	1.0	Undeter...	1.1	SP, LS

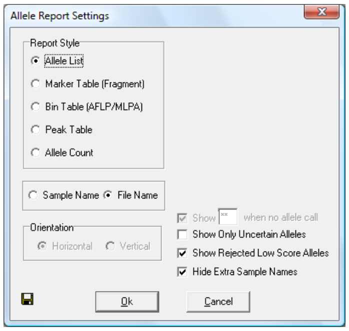
Chapter 6 Reports and Printing

Chapter 6 Reports and Printing
Report Table
Print Report
Save Project

Report Table

The general features of the *Report Table* were outlined in **Chapter 3 Main Analysis Overview**. Here we will discuss and give examples of each *Report Style* available in the *Report Table*.

Allele List



Allele Report Settings

Report Style
● Allele List
○ Marker Table (Fragment)
○ Bin Table (AFLP/MLPA)
○ Peak Table
○ Allele Count

○ Sample Name ● File Name

Orientation
● Horizontal ○ Vertical

☑ Show when no allele call
☐ Show Only Uncertain Alleles
☑ Show Rejected Low Score Alleles
☑ Hide Extra Sample Names

The *Allele List Report Style* displays the basepair size (or Allele Label, if a Panel is applied) of the called peaks. The sample names are listed in rows in the far left column and peaks are numbered in columns at the top of the table. *Allele List* is the default *Report Style* when **SNPlex** *Analysis Type* is selected.

Features

Naming Options

Report each sample by sample name or by file name in the allele report

Show Only Uncertain Alleles

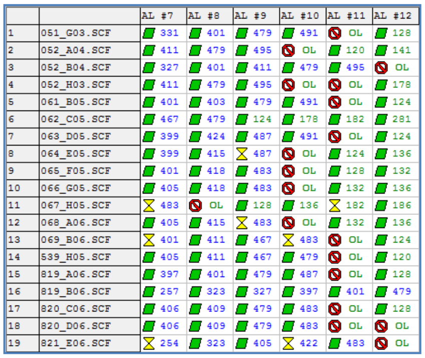
When selected, displays only the peaks with Quality ranks of Check (yellow) and Undetermined (red).

Show Rejected Low Score Alleles

When selected, the peaks with peak scores below the Run Wizard *Additional Settings Allele Evaluation Peak Score* **Reject** setting will be displayed in the table.

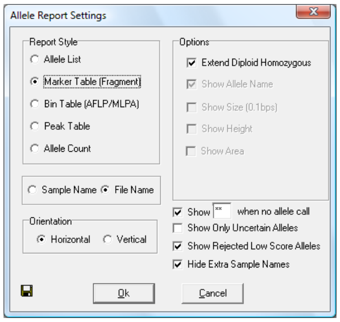
Hide Extra Sample Names

When data is displayed in *Vertical Orientation*, the sample names are repeated for each row of data that the sample is associated with. If *Hide Extra Sample Names* is selected, then the sample name will only appear once in the first of the rows it is associated with.



		AL #7	AL #8	AL #9	AL #10	AL #11	AL #12
1	051_G03.SCF	▰ 331	▰ 401	▰ 479	▰ 491	⊘ OL	▰ 128
2	052_A04.SCF	▰ 411	▰ 479	▰ 495	⊘ OL	▰ 120	▰ 141
3	052_B04.SCF	▰ 327	▰ 401	▰ 411	▰ 479	▰ 495	⊘ OL
4	052_H03.SCF	▰ 411	▰ 479	▰ 495	⊘ OL	⊘ OL	▰ 178
5	061_B05.SCF	▰ 401	▰ 403	▰ 479	▰ 491	⊘ OL	▰ 124
6	062_C05.SCF	▰ 467	▰ 479	▰ 124	▰ 178	▰ 182	▰ 281
7	063_D05.SCF	▰ 399	▰ 424	▰ 487	▰ 491	⊘ OL	▰ 124
8	064_E05.SCF	▰ 399	▰ 415	⧗ 487	⊘ OL	▰ 124	▰ 136
9	065_F05.SCF	▰ 401	▰ 418	▰ 483	⊘ OL	▰ 128	▰ 132
10	066_G05.SCF	▰ 405	▰ 418	▰ 483	⊘ OL	▰ 132	▰ 136
11	067_H05.SCF	⧗ 483	⊘ OL	▰ 128	▰ 136	⧗ 182	▰ 186
12	068_A06.SCF	▰ 405	▰ 415	⧗ 483	⊘ OL	▰ 132	▰ 136
13	069_B06.SCF	⧗ 401	▰ 411	▰ 467	⧗ 483	⊘ OL	▰ 124
14	539_H05.SCF	▰ 405	▰ 411	▰ 467	▰ 479	⊘ OL	▰ 120
15	819_A06.SCF	▰ 397	▰ 401	▰ 479	▰ 487	⊘ OL	▰ 128
16	819_B06.SCF	▰ 257	▰ 323	▰ 327	▰ 397	▰ 401	▰ 479
17	820_C06.SCF	▰ 406	▰ 409	▰ 479	▰ 483	⊘ OL	▰ 128
18	820_D06.SCF	▰ 406	▰ 409	▰ 479	▰ 483	⊘ OL	⊘ OL
19	821_E06.SCF	⧗ 254	▰ 323	▰ 405	⧗ 422	▰ 483	⊘ OL

Marker Table (Fragment)



Allele Report Settings

Report Style
○ Allele List
● Marker Table (Fragment)
○ Bin Table (AFLP/MLPA)
○ Peak Table
○ Allele Count

○ Sample Name ● File Name

Orientation
● Horizontal ○ Vertical

Options
☑ Extend Diploid Homozygous
☑ Show Allele Name
☐ Show Size (0.1bps)
☐ Show Height
☐ Show Area

☑ Show when no allele call
☐ Show Only Uncertain Alleles
☑ Show Rejected Low Score Alleles
☑ Hide Extra Sample Names

The *Marker Table (Fragment) Report Style* displays the Quality rank and Allele Label of each called peak. Samples are listed in rows in the far left column and Panel Marker names indicate the columns at the top of the table.

Marker Table (Fragment) is the default *Report Style* when **Fragment Animal, Fragment Plant,** and **HID** *Analysis Types* are selected.

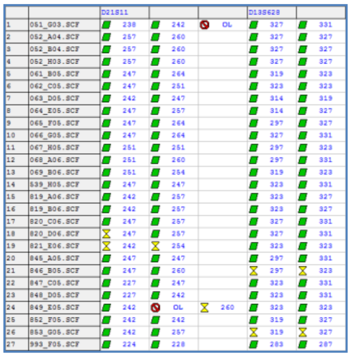
NOTE: *Marker Table (Fragment)* requires that a Panel is applied to the data. **See Chapter 5 Panel Editor.**

Features

Options

Extend Diploid Homozygous: Repeats the same Allele Label in the second allele position of the marker when only one peak is detected in the marker. Only active when the *Edit Panel Ploidy* option is set to *2-Diploid*.

Show Allele Name/Size (0.1bp)/Height/Area: *Allele Name* is displayed in the *Report Table* by default regardless of table *Orientation*. Select to display *Size, Height,* and/or *Area* of the peak all within the same cell. Parentheses separate the peak statistics from the *Allele Name*. Only enabled when *Vertical Orientation* is selected.



		D21S11			D13S628	
1	051_G03.SCF	▰ 238	▰ 242	⊘ OL	▰ 327	▰ 331
2	052_A04.SCF	▰ 257	▰ 260		▰ 327	▰ 327
3	052_B04.SCF	▰ 257	▰ 260		▰ 327	▰ 327
4	052_H03.SCF	▰ 257	▰ 260		▰ 327	▰ 327
5	061_B05.SCF	▰ 247	▰ 264		▰ 319	▰ 323
6	062_C05.SCF	▰ 247	▰ 251		▰ 323	▰ 323
7	063_D05.SCF	▰ 242	▰ 247		▰ 314	▰ 319
8	064_E05.SCF	▰ 247	▰ 257		▰ 314	▰ 327
9	065_F05.SCF	▰ 247	▰ 264		▰ 297	▰ 327
10	066_G05.SCF	▰ 247	▰ 264		▰ 327	▰ 331
11	067_H05.SCF	▰ 251	▰ 251		▰ 297	▰ 323
12	068_A06.SCF	▰ 251	▰ 260		▰ 297	▰ 331
13	069_B06.SCF	▰ 251	▰ 254		▰ 319	▰ 323
14	539_H05.SCF	▰ 247	▰ 247		▰ 323	▰ 331
15	819_A06.SCF	▰ 242	▰ 257		▰ 323	▰ 327
16	819_B06.SCF	▰ 242	▰ 257		▰ 323	▰ 327
17	820_C06.SCF	▰ 247	▰ 257		▰ 327	▰ 331
18	820_D06.SCF	⧗ 247	▰ 257		▰ 327	▰ 331
19	821_E06.SCF	⧗ 242	⧗ 254		▰ 323	▰ 323
20	845_A05.SCF	▰ 247	▰ 247		▰ 297	▰ 331
21	846_B05.SCF	▰ 247	▰ 260		⧗ 297	⧗ 323
22	847_C05.SCF	▰ 227	▰ 247		▰ 323	▰ 331
23	848_D05.SCF	▰ 227	▰ 242		▰ 323	▰ 331
24	849_E05.SCF	▰ 242	⊘ OL	⧗ 260	▰ 323	▰ 323
25	852_F05.SCF	▰ 242	▰ 242		▰ 319	▰ 327
26	853_G05.SCF	▰ 242	▰ 257		⧗ 319	⧗ 327
27	993_F05.SCF	▰ 224	▰ 228		▰ 283	▰ 287

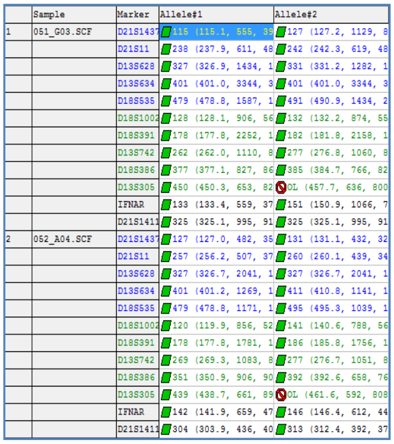
Orientation

Horizontal: Sample names appear on the left in rows and Markers appear at the top in columns.
Vertical: Sample names appear in the far left column in rows with Markers listed in the second column. Alleles in order of basepair size appear at the top in columns.

*Show ** when no allele call:* **When selected this option allows the user to specify symbol or short word, such as Null, when there are no peaks in a marker. If deselected the cell in the report would be empty.**

Show Only Uncertain Alleles: When selected, displays only the peaks with Quality ranks of *Check* (yellow) and *Undetermined* (red).

Show Rejected Low Score Alleles: When selected, the peaks with peak scores below the Run Wizard *Additional Settings Allele Evaluation Peak Score* **Reject** setting will be displayed in the table.



	Sample	Marker	Allele#1	Allele#2
1	051_G03.SCF	D21S1437	🟩115 (115.1, 555, 39	🟩127 (127.2, 1129, 8
		D21S11	🟩238 (237.9, 611, 48	🟩242 (242.3, 619, 48
		D13S628	🟩327 (326.9, 1434, 1	🟩331 (331.2, 1282, 1
		D13S634	🟩401 (401.0, 3344, 3	🟩401 (401.0, 3344, 3
		D18S535	🟩479 (478.8, 1587, 1	🟩491 (490.9, 1434, 2
		D18S1002	🟩128 (128.1, 906, 56	🟩132 (132.2, 874, 55
		D18S391	🟩178 (177.8, 2252, 1	🟩182 (181.8, 2158, 1
		D13S742	🟩262 (262.0, 1110, 8	🟩277 (276.8, 1060, 8
		D18S386	🟩377 (377.1, 827, 86	🟩385 (384.7, 766, 82
		D13S305	🟩450 (450.3, 653, 82	⃠OL (457.7, 636, 800
		IFNAR	🟩133 (133.4, 559, 37	🟩151 (150.9, 1066, 7
		D21S1411	🟩325 (325.1, 995, 91	🟩325 (325.1, 995, 91
2	052_A04.SCF	D21S1437	🟩127 (127.0, 482, 35	🟩131 (131.1, 432, 32
		D21S11	🟩257 (256.2, 507, 37	🟩260 (260.1, 439, 34
		D13S628	🟩327 (326.7, 2041, 1	🟩327 (326.7, 2041, 1
		D13S634	🟩401 (401.2, 1269, 1	🟩411 (410.8, 1141, 1
		D18S535	🟩479 (478.8, 1171, 1	🟩495 (495.3, 1039, 1
		D18S1002	🟩120 (119.9, 856, 52	🟩141 (140.6, 788, 56
		D18S391	🟩178 (177.8, 1781, 1	🟩186 (185.8, 1756, 1
		D13S742	🟩269 (269.3, 1083, 8	🟩277 (276.7, 1051, 8
		D18S386	🟩351 (350.9, 906, 90	🟩392 (392.6, 658, 76
		D13S305	🟩439 (438.7, 661, 89	⃠OL (461.6, 592, 808
		IFNAR	🟩142 (141.9, 659, 47	🟩146 (146.4, 612, 44
		D21S1411	🟩304 (303.9, 436, 40	🟩313 (312.4, 392, 37

Hide Extra Sample Names: When data is displayed in *Vertical Orientation*, the sample names are repeated for each row of data that the sample is associated with. If *Hide Extra Sample Names* is selected, then the sample name will only appear once in the first of the rows it is associated with.

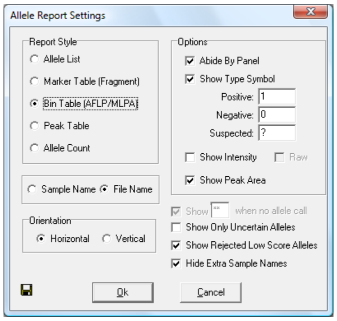
Bin Table (AFLP/MLPA)

If a peak is detected in at least one sample, the *Bin Table Report Style* will report the presence or absence of a peak at that position for the rest of the samples in the dataset. *Bin Table* is the default *Report Style* when **AFLP, MLPA**, or **SNaPshot** *Analysis Types* are selected.

Features

Options

Abide By Panel: When selected, the table will show only called alleles within Panel Marker ranges. This option is only active when a Panel is applied to the data.
Show Type Symbol: Enter values to indicate the presence of a peak at the position (*Positive*), the absence of a peak at the position (*Negative*), and a Check or Undetermined Quality rank at the position (*Suspected*).
Show Intensity (Raw): Displays the peak intensity (RFU) value for all *Positive* and *Suspected* peak positions. A "0" value is given to *Negative* positions. Selecting *Raw* will show the peak intensity values for all positions including *Negative* positions.
Show Peak Area: Displays the peak area value for all *Positive* and *Suspected* peak positions. Dollar signs "$" separate values if more than one display option is selected.



Allele Report Settings

Report Style
○ Allele List
○ Marker Table (Fragment)
● Bin Table (AFLP/MLPA)
○ Peak Table
○ Allele Count

○ Sample Name ● File Name

Orientation
● Horizontal ○ Vertical

Options
☑ Abide By Panel
☑ Show Type Symbol
Positive: 1
Negative: 0
Suspected: ?
☐ Show Intensity ☐ Raw
☑ Show Peak Area

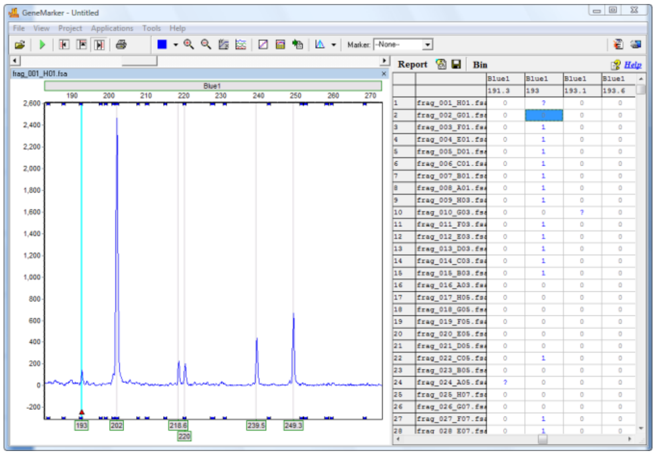
☑ Show ** when no allele call
☐ Show Only Uncertain Alleles
☑ Show Rejected Low Score Alleles
☑ Hide Extra Sample Names

Orientation

Horizontal: Sample names appear on the left in rows and Markers appear at the top in columns.
Vertical: Sample names appear in the far left column in rows. Markers and Alleles, in order of basepair size, appear at the top in columns.

Show Only Uncertain Alleles: When selected, displays only the peaks with Quality ranks of *Check* (yellow) and *Undetermined* (red).

Show Rejected Low Score Alleles: When selected, the peaks with peak scores below the Run Wizard *Additional Settings Allele Evaluation Peak Score* **Reject** setting will be displayed in the table.



		Blue1	Blue1	Blue1	Blue1
		191.3	193	193.1	193.6
1	frag_001_H01.fs	0	?	0	0
2	frag_002_G01.fs	0	0	0	0
3	frag_003_F01.fs	0	1	0	0
4	frag_004_E01.fs	0	1	0	0
5	frag_005_D01.fs	0	1	0	0
6	frag_006_C01.fs	0	1	0	0
7	frag_007_B01.fs	0	1	0	0
8	frag_008_A01.fs	0	1	0	0
9	frag_009_H03.fs	0	1	0	0
10	frag_010_G03.fs	0	0	?	0
11	frag_011_F03.fs	0	1	0	0
12	frag_012_E03.fs	0	1	0	0
13	frag_013_D03.fs	0	1	0	0
14	frag_014_C03.fs	0	1	0	0
15	frag_015_B03.fs	0	1	0	0
16	frag_016_A03.fs	0	0	0	0
17	frag_017_H05.fs	0	0	0	0
18	frag_018_G05.fs	0	0	0	0
19	frag_019_F05.fs	0	0	0	0
20	frag_020_E05.fs	0	0	0	0
21	frag_021_D05.fs	0	0	0	0
22	frag_022_C05.fs	0	1	0	0
23	frag_023_B05.fs	0	0	0	0
24	frag_024_A05.fs	?	0	0	0
25	frag_025_H07.fs	0	0	0	0
26	frag_026_G07.fs	0	0	0	0
27	frag_027_F07.fs	0	1	0	0
28	frag_028_E07.fs	0	1	0	0

Hide Extra Sample Names: When data is

displayed in *Vertical Orientation*, the sample names are repeated for each row of data that the sample is associated with. If *Hide Extra Sample Names* is selected, then the sample name will only appear once in the first of the rows it is associated with.

Additional Functions

Allele Editing Options

The *Bin Table Report Style* offers additional options when a cell in the table is right-clicked.

Insert a Peak at this Bin Site

To indicate the presence of a peak at a position when it has been labeled with a *Negative Type Symbol*, right-click the cell and select *Insert a Peak at this Bin Site*. The *Negative Type Symbol* will change to a *Positive* or *Suspected Type Symbol* depending on the Quality rank of the peak. (**Hot Key** **= INS**)

Delete

To indicate the absence of a peak at a position that has been labeled with a *Positive* or *Suspect Type Symbol*, right-click the peak cell and select *Delete.* The *Type Symbol* will change to *Negative*. (**Hot Key** **= DEL**)

Confirm

To indicate the peak present at the position is truly a peak, right-click the peak cell and select *Confirm Peaks*. Only peaks centered within a Panel *Bin* will change from Suspect Type Symbol to *Positive Type Symbol* when confirmed. Once a peak is confirmed, it cannot be unconfirmed, only deleted. (**Hot Key** **= CTRL+M**)

Delete Bin Columns

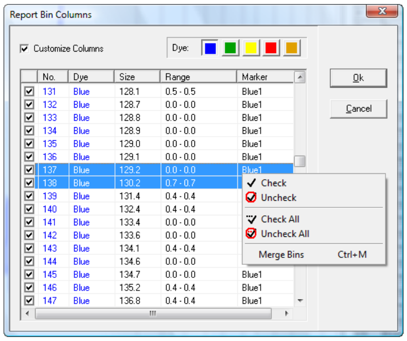
To completely eliminate an entire column in the *Report Table*, left-click any cell within the column then right-click the cell and select *Delete Bin Columns*. When *Vertical Orientation* is selected, the *Report Table* rows which contain the *Bin* information will be deleted (not the columns which contain the sample information).

Binning Options

To adjust which Bins are displayed and to merge Bins in the *Report Table*, click the **Bin** icon in the toolbar of the *Report Table*. The *Report Bin Columns* box will appear.

Display Bins

By default, all Bins will be selected with a checkmark at the beginning of the row. Individually deselect Bins for exclusion from the *Report Table* by single left-clicking the checkmark box. To deselect all, right-click any cell in the *Report Bin Columns* box and select *Uncheck All*. To deselect only a few Bins, left-click a cell to highlight the row then hold **CTRL** or **SHIFT** key and select additional rows. Next, right-click and select *Check* or *Uncheck* to include or exclude the Bins, respectively. Click **OK** in the *Report Bin Columns* box when finished and only the Bins with checkmarks will be displayed in the *Report Table.*



☑ Customize Columns

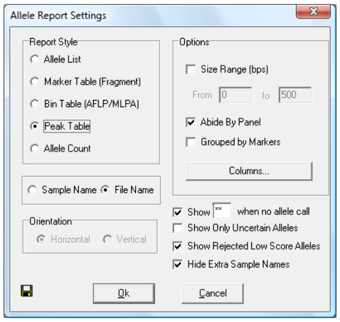
	No.	Dye	Size	Range	Marker
☑	131	Blue	128.1	0.5 - 0.5	Blue1
☑	132	Blue	128.7	0.0 - 0.0	Blue1
☑	133	Blue	128.8	0.0 - 0.0	Blue1
☑	134	Blue	128.9	0.0 - 0.0	Blue1
☑	135	Blue	129.0	0.0 - 0.0	Blue1
☑	136	Blue	129.1	0.0 - 0.0	Blue1
☑	137	Blue	129.2	0.0 - 0.0	Blue1
☑	138	Blue	130.2	0.7 - 0.7	
☑	139	Blue	131.4	0.4 - 0.4	
☑	140	Blue	132.4	0.4 - 0.4	
☑	141	Blue	133.4	0.0 - 0.0	
☑	142	Blue	133.6	0.0 - 0.0	
☑	143	Blue	134.1	0.4 - 0.4	
☑	144	Blue	134.6	0.0 - 0.0	
☑	145	Blue	134.7	0.0 - 0.0	Blue1
☑	146	Blue	135.2	0.4 - 0.4	Blue1
☑	147	Blue	136.8	0.4 - 0.4	Blue1

Merge Bins

To make two or more Bins become one Bin, single left-click a row to highlight it. Next, hold down **SHIFT** key to select additional rows. Right-click the highlighted rows and select *Merge Bins*. (**Hot Key** **= CTRL+M**) Click **OK** in the *Report Bin Columns* box when finished, and the selected Bins will be averaged together. Only Bins immediately adjacent to one another may be selected for merging. Only the height and area for the first peak in the new merged Bin will be reported.

Peak Table

The *Peak Table Report Style* displays user-defined peak statistics. Sample names are displayed in the far left column in rows and the Marker names are in the column adjacent to the sample names. In columns at the top of the table are the selected peak statistic information labels. *Peak Table* is available for all *Analysis Types*.



Report Style
○ Allele List
○ Marker Table (Fragment)
○ Bin Table (AFLP/MLPA)
● Peak Table
○ Allele Count

○ Sample Name ● File Name

Orientation
● Horizontal ○ Vertical

Options
☐ Size Range (bps)
From 0 to 500
☑ Abide By Panel
☐ Grouped by Markers

☑ Show ** when no allele call
☐ Show Only Uncertain Alleles
☑ Show Rejected Low Score Alleles
☑ Hide Extra Sample Names

The column options available in the *Peak Table Report Style* are similar to the options available in the *Peak Table* that appears below the *Electropherograms*. **See Chapter 3 Main Analysis Overview** for column option definitions.

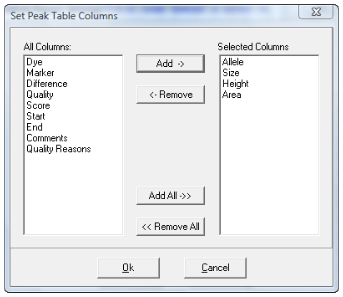
Features

Options

Size Range (bps): When selected, allows the user to define a specific basepair range. Only the peaks within the range will be displayed within the *Report Table*.
Abide By Panel: When selected, the table will show only called alleles within Panel Marker ranges. This option is only active when a Panel is applied to the data.
Grouped by Markers: When selected, alleles within the Marker will be listed one after the other in the columns at the top of the table. When de-selected, each allele will be represented by a row so that the Marker name may be listed several times according to the number of alleles in the Marker. This option is only active when a Panel is applied to the data.

Columns

Click the **Columns** button to open the *Set Peak Table Columns* box. All column options are listed in the *All Columns* field on the left. The columns currently being displayed in the *Report Table* are listed in the *Selected Columns* field on the right.



All Columns:
Dye
Marker
Difference
Quality
Score
Start
End
Comments
Quality Reasons

Selected Columns
Allele
Size
Height
Area

Selecting Columns

Single left-click options in the *All Columns* field and click the **Add** button to add the column option to the *Selected Columns* field. Hold down **CTRL** or **SHIFT** key to select multiple options then click **Add**. Click the **Add All** button to move all the options in the *All Columns* field to the *Selected Columns* field.

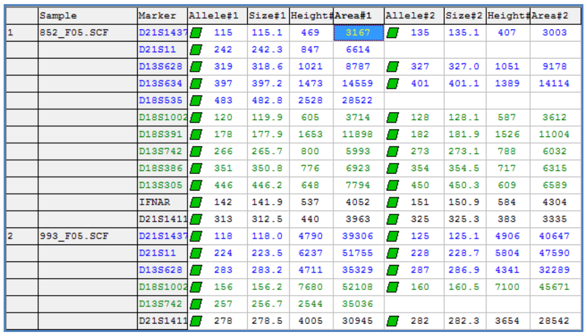
De-selecting Columns

Single left-click options in the *Selected Columns* field and click **Remove** to move the column option to the *All Columns* field. Hold down **CTRL** or **SHIFT** key to select multiple options then click **Remove**. Click the **Remove All** button to move all the options in the *Selected Columns* field to the *All Columns* field.

Click **OK** in the *Set Peak Table Columns* box and the *Allele Report Settings* box when finished. The options in the *Selected Column* field will be displayed along the top of the table in columns.

Show ** when no allele call: **When selected this option allows the user to specify symbol or short word, such as Null, when there are no peaks in a marker. If deselected the cell in the report would be empty.**

Show Only Uncertain Alleles: When selected, displays only the peaks with Quality ranks of *Check* (yellow) and *Undetermined* (red).



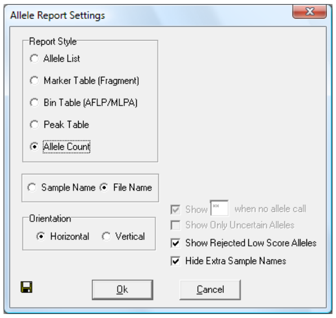
	Sample	Marker	Allele#1	Size#1	Height#	Area#1	Allele#2	Size#2	Height#	Area#2
1	852_F05.SCF	D21S1437	🟩 115	115.1	469	3167	🟩 135	135.1	407	3003
		D21S11	🟩 242	242.3	847	6614				
		D13S628	🟩 319	318.6	1021	8787	🟩 327	327.0	1051	9178
		D13S634	🟩 397	397.2	1473	14559	🟩 401	401.1	1389	14114
		D18S535	🟩 483	482.8	2528	28522				
		D18S1002	🟩 120	119.9	605	3714	🟩 128	128.1	587	3612
		D18S391	🟩 178	177.9	1653	11898	🟩 182	181.9	1526	11004
		D13S742	🟩 266	265.7	800	5993	🟩 273	273.1	788	6032
		D18S386	🟩 351	350.8	776	6923	🟩 354	354.5	717	6315
		D13S305	🟩 446	446.2	648	7794	🟩 450	450.3	609	6589
		IFNAR	🟩 142	141.9	537	4052	🟩 151	150.9	584	4304
		D21S1411	🟩 313	312.5	440	3963	🟩 325	325.3	383	3335
2	993_F05.SCF	D21S1437	🟩 118	118.0	4790	39306	🟩 125	125.1	4906	40647
		D21S11	🟩 224	223.5	6237	51755	🟩 228	228.7	5804	47590
		D13S628	🟩 283	283.2	4711	35329	🟩 287	286.9	4341	32289
		D18S1002	🟩 156	156.2	7680	52108	🟩 160	160.5	7100	45671
		D13S742	🟩 257	256.7	2544	35036				
		D21S1411	🟩 278	278.5	4005	30945	🟩 282	282.3	3654	28542

Show Rejected Low Score Alleles: When selected, the peaks with peak scores below the Run Wizard *Additional Settings Allele Evaluation Peak Score* **Reject** setting will be displayed in the table.

Hide Extra Sample Names: When data is displayed in *Vertical Orientation*, the sample names are repeated for each row of data that the sample is associated with. If *Hide Extra Sample Names* is selected, then the sample name will only appear once in the first of the rows it is associated with.

Allele Count

The *Allele Count Report Style* displays the number of alleles present in the Panel Marker. Sample names are listed in rows in the left column and Markers are listed along the top row in columns. A *Total Number* column lists the number of peaks detected in the sample.
NOTE: *Allele Count* requires that a Panel is applied to the data. **See Chapter 5 Panel Editor.**



Report Style
○ Allele List
○ Marker Table (Fragment)
○ Bin Table (AFLP/MLPA)
○ Peak Table
● Allele Count

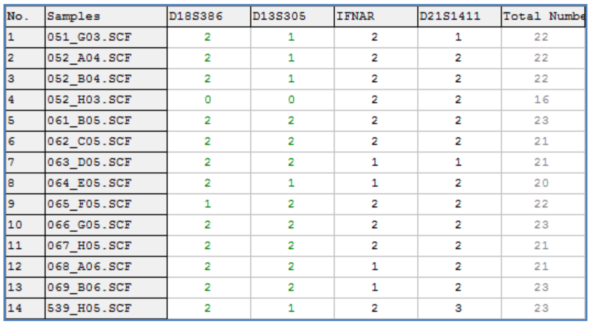
○ Sample Name ● File Name

Orientation
● Horizontal ○ Vertical

☑ Show ** when no allele call
☐ Show Only Uncertain Alleles
☑ Show Rejected Low Score Alleles
☑ Hide Extra Sample Names

Features

Orientation



No.	Samples	D18S386	D13S305	IFNAR	D21S1411	Total Numbe
1	051_G03.SCF	2	1	2	1	22
2	052_A04.SCF	2	1	2	2	22
3	052_B04.SCF	2	1	2	2	22
4	052_H03.SCF	0	0	2	2	16
5	061_B05.SCF	2	2	2	2	23
6	062_C05.SCF	2	2	2	2	21
7	063_D05.SCF	2	2	1	1	21
8	064_E05.SCF	2	1	1	2	20
9	065_F05.SCF	1	2	2	2	22
10	066_G05.SCF	2	2	2	2	23
11	067_H05.SCF	2	2	2	2	21
12	068_A06.SCF	2	2	1	2	21
13	069_B06.SCF	2	2	1	2	23
14	539_H05.SCF	2	1	2	3	23

Horizontal: Sample names appear on the left in rows and Markers appear at the top in columns.
Vertical: Markers appear on the left in rows and sample names appear at the top in columns.

Show Rejected Low Score Alleles: When selected, the peaks with peak scores below the Run Wizard *Additional Settings Allele Evaluation Peak Score* **Reject** setting will be displayed in the table.

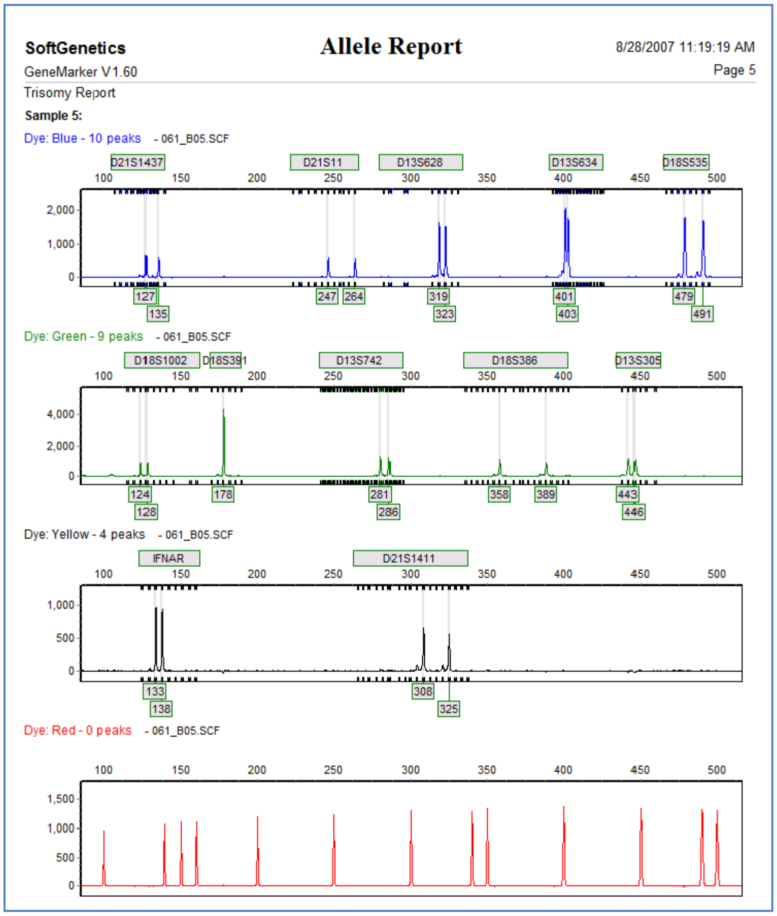
Hide Extra Sample Names: This feature is not active for *Allele Count Report Style*.

Print Report

The GeneMarker *Print Report* displays *Electropherogram* and/or *Peak Table* information for all or selected samples in a dataset. To access the *Print Report*, go to *Project* → *Print Report* OR click the **Print Report** icon in the *Main Analysis* window. The *Print Report* options box will appear. Select desired settings and click **Preview** to view the *Print Report* before printing or click **OK** to begin printing without previewing the report.

NOTE: The *View* → *Preference* → *Display Settings* options will affect how the *Print Report* is displayed.

GeneMarker Print Report

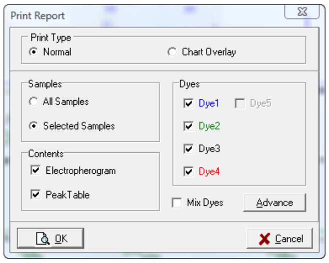


SoftGenetics **Allele Report** 8/28/2007 11:19:19 AM
GeneMarker V1.60 Page 5
Trisomy Report
Sample 5:
Dye: Blue - 10 peaks - 061_B05.SCF
Dye: Green - 9 peaks - 061_B05.SCF
Dye: Yellow - 4 peaks - 061_B05.SCF
Dye: Red - 0 peaks - 061_B05.SCF

D21S1437
D21S11
D13S628
D13S634
D18S535
100
150
200
250
300
350
400
450
500
2,000
1,000
0
127
135
247
264
319
323
401
403
479
491
D18S1002
D18S391
D13S742
D18S386
D13S305
4,000
124
128
178
281
286
358
389
443
446
IFNAR
D21S1411
500
133
138
308
325
1,500

Report Content Options

The basic printing options allow the user to choose the *Print Type, Samples* to print, *Dyes* to include, and *Content* options. Each electropherogram will be automatically labeled with its respective sample file name in the printed report. The *Advanced* button provides more options.


Print Report
Print Type: ● Normal ○ Chart Overlay
Samples: ○ All Samples ● Selected Samples
Contents: ☑ Electropherogram ☑ PeakTable
Dyes: ☑ Dye1 ☐ Dye5 ☑ Dye2 ☑ Dye3 ☑ Dye4
☐ Mix Dyes

Print Type

Normal: All *Print Report* options are available when *Normal Print Type* is selected.

Chart Overlay: Prints only the *Electropherogram* with the report.

Samples

All Samples: Prints all the samples in the project.

Selected Samples: Prints only those sample files that have been selected in the *Main Analysis* window *Sample File Tree*.

Contents

Electropherogram: Prints the peak trace for each dye color and sample selected.

NOTE: The zoom setting of the *Electropherogram* in the *Main Analysis* window will be represented in the *Print Report*. Zoom out fully to include all peaks in the *Print Report*.

Peak Table: Prints the *Peak Table* for each dye color below the dye color's electropherogram trace.

NOTE: If neither *Electropherogram* nor *Peak Table* were selected, the *Print Report* will contain a list of each dye color selected for each sample selected and the allele count within each dye color.

Dyes

Dye 1-4: Click the checkbox to include the dye color in the *Print Report*.

Mix Dyes: Prints all selected dye colors on one electropherogram.

Advanced Options

Print Project Comments: Includes the *Project Comments* at the top of the *Print Report*. Select *Each Page* option to display the *Project Comments* on each page in the report. Select Word Wrap if comments require more than one line on the page.

Label Dyes & Peak Numbers: Labels dye color with number of peaks for each electropherogram.

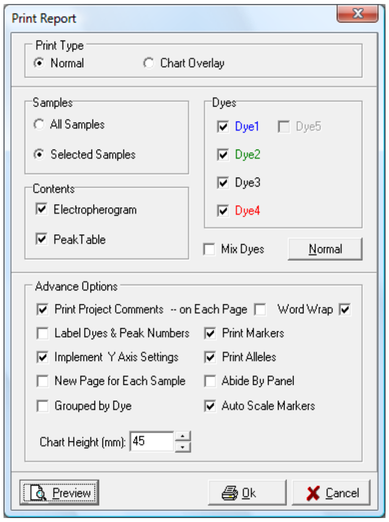
Implement Y Axis Settings: Prints the report using the Y-axis settings the user selected in the *Main Analysis* window *Set Axis* icon.

Chart Height (mm): Specify the size of the printed electropherograms (Minimum = 10mm, maximum = 100mm).

Print Markers: The Marker label bars appear above the electropherogram.

Print Alleles: The Allele Labels appear below the electropherogram.

Abide by Panel: Prints only alleles within a Panel. Alleles that are outside the Panel are not included in the printed report.


Print Report
Print Type: ● Normal ○ Chart Overlay
Samples: ○ All Samples ● Selected Samples
Contents: ☑ Electropherogram ☑ PeakTable
Dyes: ☑ Dye1 ☐ Dye5 ☑ Dye2 ☑ Dye3 ☑ Dye4
☐ Mix Dyes
Advance Options
☑ Print Project Comments -- on Each Page ☐ Word Wrap ☑
☐ Label Dyes & Peak Numbers ☑ Print Markers
☑ Implement Y Axis Settings ☑ Print Alleles
☐ New Page for Each Sample ☐ Abide By Panel
☐ Grouped by Dye ☑ Auto Scale Markers
Chart Height (mm): 45

New Page for Each Sample: Prints a new page for each sample instead of continuing on the same page as the previous sample.

Auto Scale Markers When selected, the RFU intensities of low peaks are adjusted to match the intensity of the highest peak in the dye color. When low peaks are increased, the intensity magnification factor is noted in the Marker (2X – 8X).

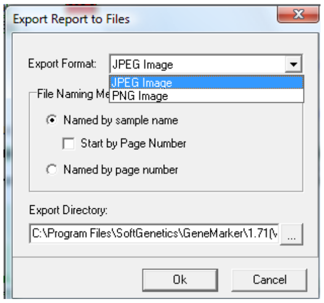
Grouped by Dye: Organizes the electropherograms in the *Print Report* such that samples are listed in order of dye color selected i.e. all samples in blue first, then all samples in green, etc.

Icons and Functions

The following icons are available in the *Print Preview* window prior to printing the *Print Report*.

Print
Opens the *Print* options box. Select a printer, the print range and the number of copies.

Export to File
Opens the *Export Report to Files* box. Save each page of the *Print Report* as an individual image file (PNG or JPEG).



Export Report to Files

Export Format: JPEG Image

File Naming Mode

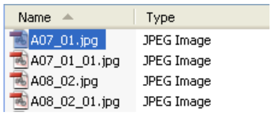
● Named by sample name

☐ Start by Page Number

○ Named by page number

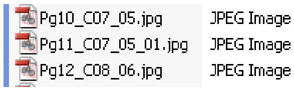
Export Directory: C:\Program Files\SoftGenetics\GeneMarker\1.71(V

Named by sample name saves each PNG or JPEG under the sample name.



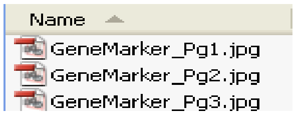
Name	Type
A07_01.jpg	JPEG Image
A07_01_01.jpg	JPEG Image
A08_02.jpg	JPEG Image
A08_02_01.jpg	JPEG Image

Start by Page Number combines the page number and the sample name for the saved file name.



Pg10_C07_05.jpg	JPEG Image
Pg11_C07_05_01.jpg	JPEG Image
Pg12_C08_06.jpg	JPEG Image

Named by page number saves each JPEG file by the page number within the report.



Name

GeneMarker_Pg1.jpg

GeneMarker_Pg2.jpg

GeneMarker_Pg3.jpg

Page Setup
Opens the *Page Setup* box. Choose the paper size, margins and orientation (*Portrait* or *Landscape*).

Content Options
Opens the *Print Report* options box. See the section above - *Report Content Options*.

Zoom to Fit
Zooms out to view the entire *Print Preview* page.

Zoom to Width
Zooms in to view the *Print Preview* page at maximum width without losing information off the screen.

100

Zoom Ratio
Enter percentage numbers to increase or decrease the zoom aspect of the *Print Preview* page.

Save Project

After a dataset is analyzed and edited, the project can be saved as a SoftGenetics GeneMarker Project (SGF). Project files contain the raw, unprocessed data files, the sample files after processing, the process parameters, and all edits. The project file does not contain any custom or modified Panels or Size Standards. To export a custom Panel, see **Chapter 5 Panel Editor**. To export a custom Size Standard, see **Chapter 4 Fragment Sizing Standards**.

NOTE: Previous versions of GeneMarker saved projects in SFP format. This format can be opened with current GeneMarker versions.

To save a project, go to *File → Save Project* in the *Main Analysis* window. The *Save Project* box will open. Select a directory and enter a project name. Click **Save**.

To re-open the project, go to *File → Open Project* in the *Main Analysis* window. The last folder accessed by GeneMarker will appear. Navigate to the directory containing the SGF (or SFP) file and click **Open**. Additionally, the last four projects that were opened by GeneMarker appear when the *File → Reopen Project* fly-out menu is selected. Click a project from the fly-out menu and it will be uploaded to GeneMarker.

Chapter 7 Special Applications

Chapter 7 Special Applications

AFLP
T-RFLP
MLPA
Human Identity
Pedigree Chart
Kinship Analysis
Database Search
Automated Pedigree Drawing
Quantitative Analysis
SNP Analysis
Microsatellite Instability
Trisomy Detection
Loss of Heterozygosity
TILLING® Analysis
Haplotype Analysis
ARMS/Comparative Analysis Cystic Fibrosis

Amplified Fragment Length Polymorphism (AFLP)

Amplified Fragment Length Polymorphism (AFLP®) is a polymerase chain reaction (PCR) based genetic fingerprinting technique developed in the early 1990s by Keygene. AFLP uses restriction enzymes to cut genomic DNA, followed by ligation of complementary double-stranded adaptors to the ends of the restriction fragments. A subset of the restriction fragments are amplified using two primers, containing amplification-selective nucleotides at their 3'-ends, complementary to the adaptor and restriction fragments. These fragments are visualized on denaturing polyacrylamide gels using either autoradiographic or fluorescence methodologies.

Procedure

Analysis procedures for AFLP analysis include uploading the data, sizing and filtering the data with Run Wizard, creating a Panel if desired, and, finally, detecting subgroups within the population. Below is an explanation of AFLP settings in Run Wizard and Panel creation for AFLP data. Subsequent sections will describe in detail how the **Allele Detection with Bin Table**, **Reference Trace Comparison**, and/or **Phylogeny Clustering Analysis** can facilitate subpopulation discovery.

AFLP Run Wizard Settings

For explanation of specific functions in Run Wizard, **see Chapter 2 General Procedure**.

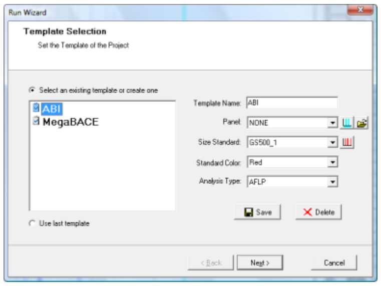
Run Wizard Template Selection

Panel: NONE OR chose custom Panel

Size Standard: User-defined

Analysis Type: AFLP

NOTE: Depending on your experiment, it may or may not be necessary to select a Panel for data analysis. The *Clustering Analysis* module is only available when a Panel is applied.



Template Selection

Set the Template of the Project

● Select an existing template or create one

ABI
MegaBACE

Template Name: ABI
Panel: NONE
Size Standard: GS500_1
Standard Color: Red
Analysis Type: AFLP

○ Use last template

Run Wizard Data Process

Peak Detection Intensity Threshold: 100

Global Percentage: 1% Max

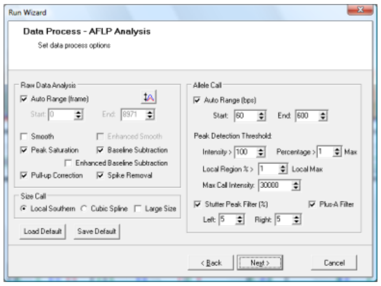
Local Percentage: 1%

Max Call Intensity: 30,000

Stutter Peak Filter: Left: 5% Right: 5%

Plus A Filter: Selected

NOTE: The correct settings for AFLP analysis should be a small global percentage plus a slightly larger or equal local percentage. It is suggested that the stutter peak filter be selected to allow the software to remove stutter peaks within 2.5 base pairs of each detected allele peak.



Data Process - AFLP Analysis

Set data process options

Raw Data Analysis
☑ Auto Range (frame)
Start: 0 End: 8971
☐ Smooth ☐ Enhanced Smooth
☑ Peak Saturation ☑ Baseline Subtraction
☐ Enhanced Baseline Subtraction
☑ Pull-up Correction ☑ Spike Removal

Size Call
● Local Southern ○ Cubic Spline ☐ Large Size

Allele Call
☑ Auto Range (bps)
Start: 60 End: 600
Peak Detection Threshold
Intensity > 100 Percentage > 1 Max
Local Region % > 1 Local Max
Max Call Intensity: 30000
☑ Stutter Peak Filter (%) ☑ Plus-A Filter
Left: 5 Right: 5

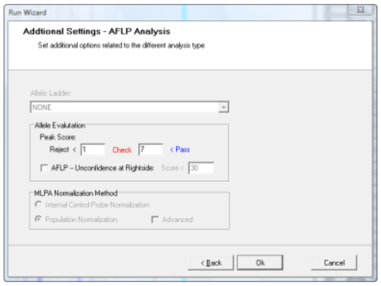
Run Wizard Additional Settings

Allele Evaluation Peak Score: Reject < 1 Check 7 < Pass

NOTE: If the Reject value is set too high, a false negative call could result. If the Reject value is set too low, then a false positive call may occur.

AFLP Unconfidence at Rightside Score: < 30

NOTE: This requires that two peaks to the right of the examined peak must have a score of at least 30 for the peak to be accepted.



Addtional Settings - AFLP Analysis

Set additional options related to the different analysis type

Allelic Ladder: NONE

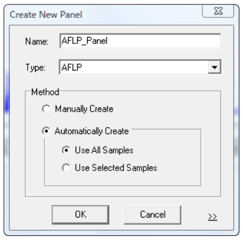
Allele Evaluation
Peak Score:
Reject < 1 Check 7 < Pass
☐ AFLP -- Unconfidence at Rightside: Score < 30

MLPA Normalization Method
○ Internal Control Probe Normalization
● Population Normalization ☐ Advanced

AFLP Panel Creation

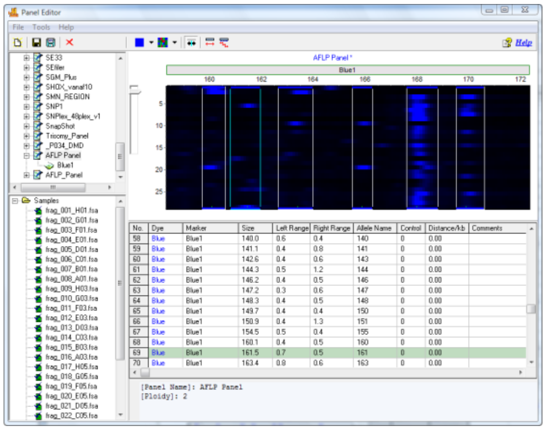
After the data has been sized and filters have been applied, go to *Tools* → *Panel Editor* to create a Panel for the dataset. For explanation of specific functions in *Panel Editor*, **see Chapter 5 Panel Editor**.

1. In *Panel Editor*, select *File* → *Create New Panel* OR click the **Create New Panel** icon
2. The *Create New Panel* box appears
3. Enter a name for the Panel and be sure **AFLP** is selected in the *Type* field
4. Select *Automatically Create* and *Use All Samples*
5. Click **OK**
6. *Panel Editor* will place one Marker in each dye color where peaks are detected
7. If only one dye color contains peaks, delete Markers created in other dye colors



Name: AFLP_Panel
Type: AFLP

Method
○ Manually Create
● Automatically Create
● Use All Samples
○ Use Selected Samples


Panel Editor
File Tools Help
Help
AFLP Panel*
Blue1
160
162
164
166
168
170
172
SE33
SEfiler
SGM_Plus
SHOX_vanaf10
SMN_REGION
SNP1
SNPlex_48plex_v1
SnapShot
Trisomy_Panel
_P034_DMD
AFLP Panel
Blue1
AFLP_Panel
Samples

No.	Dye	Marker	Size	Left Range	Right Range	Allele Name	Control	Distance/kb	Comments
58	Blue	Blue1	140.0	0.6	0.4	140	0	0.00	
59	Blue	Blue1	141.1	0.4	0.8	141	0	0.00	
60	Blue	Blue1	142.6	0.4	0.6	143	0	0.00	
61	Blue	Blue1	144.3	0.5	1.2	144	0	0.00	
62	Blue	Blue1	146.2	0.4	0.5	146	0	0.00	
63	Blue	Blue1	147.2	0.3	0.6	147	0	0.00	
64	Blue	Blue1	148.3	0.4	0.5	148	0	0.00	
65	Blue	Blue1	149.7	0.4	0.4	150	0	0.00	
66	Blue	Blue1	150.9	0.4	1.3	151	0	0.00	
67	Blue	Blue1	154.5	0.5	0.4	155	0	0.00	
68	Blue	Blue1	160.1	0.4	0.5	160	0	0.00	
69	Blue	Blue1	161.5	0.7	0.5	161	0	0.00	
70	Blue	Blue1	163.4	0.8	0.6	163	0	0.00	

```
[Panel Name]: AFLP Panel
[Ploidy]: 2
```

8. Click the **Trace Mode** icon until the *Gel Image* view appears in the *Trace Overlay* frame
9. Hold down **SHIFT** key and left-click and drag Bin edges to adjust the Bin range or drag the entire Bin to a new position
10. Right-click and select *Add/Delete Allele* to add and remove Bins from the Panel
11. After editing, select *File* → *Save Changes* (**Hot Key** = **CTRL+S**) OR click **Save Changes** icon
12. Exit the *Panel Editor*
13. Click the **Run Process** icon in the *Main Analysis* window
14. Select the newly created Panel from the Run Wizard *Template Selection Panel* drop-down menu
15. Proceed through Run Wizard and click **OK** in the *Data Process* box
16. The Panel has been applied

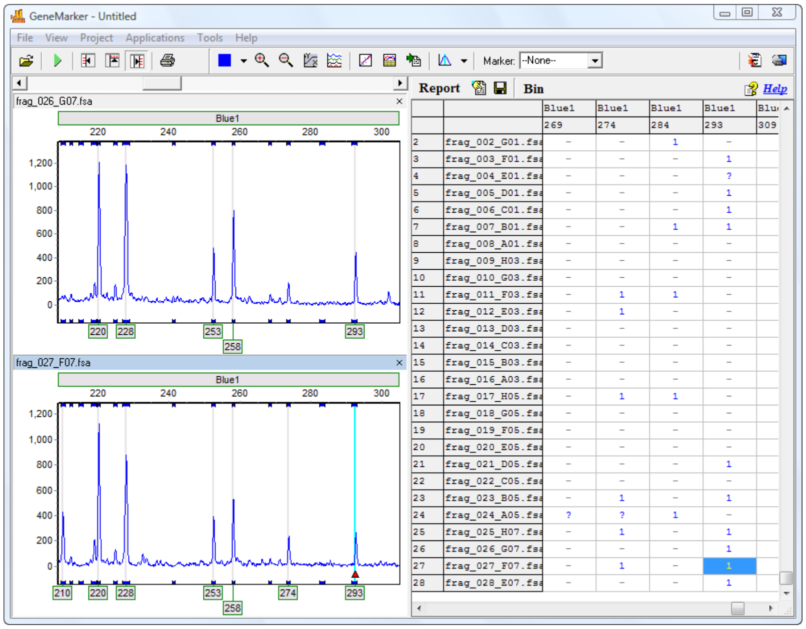
What to Expect

Three options are available for analyzing AFLP data: **Allele Detection with Bin Table, Trace Comparison**, and **Phylogeny Clustering Analysis**. The following sections describe these features in detail.

Allele Detection (Bin Table)

The *Main Analysis* window of GeneMarker is used for AFLP data analysis. The *Report Style* associated with the AFLP *Analysis Type* is *Bin Table*. The *Bin Table* is useful for identifying allele positions common in the dataset and also readily displays outlier peaks. The *Bin Table* can be exported as an Excel (.xls) or tab-delimited Text (.txt) file. **See Chapter 3 Main Analysis Overview** for *Report Table* operations and **See Chapter 6 Reports and Printing** for *Bin Table* options.

AFLP Analysis and Bin Table


GeneMarker - Untitled
File View Project Applications Tools Help
Marker: --None--
frag_026_G07.fsa
Blue1
220
240
260
280
300
1,200
1,000
800
600
400
200
0
220
228
253
258
293
frag_027_F07.fsa
Blue1
220
240
260
280
300
1,200
1,000
800
600
400
200
0
210
220
228
253
258
274
293
Report
Bin
Help

		Blue1	Blue1	Blue1	Blue1	Blu
		269	274	284	293	309
2	frag_002_G01.fsa	–	–	1	–	
3	frag_003_F01.fsa	–	–	–	1	
4	frag_004_E01.fsa	–	–	–	?	
5	frag_005_D01.fsa	–	–	–	1	
6	frag_006_C01.fsa	–	–	–	1	
7	frag_007_B01.fsa	–	–	1	1	
8	frag_008_A01.fsa	–	–	–	–	
9	frag_009_H03.fsa	–	–	–	–	
10	frag_010_G03.fsa	–	–	–	–	
11	frag_011_F03.fsa	–	1	1	–	
12	frag_012_E03.fsa	–	1	–	–	
13	frag_013_D03.fsa	–	–	–	–	
14	frag_014_C03.fsa	–	–	–	–	
15	frag_015_B03.fsa	–	–	–	–	
16	frag_016_A03.fsa	–	–	–	–	
17	frag_017_H05.fsa	–	1	1	–	
18	frag_018_G05.fsa	–	–	–	–	
19	frag_019_F05.fsa	–	–	–	–	
20	frag_020_E05.fsa	–	–	–	–	
21	frag_021_D05.fsa	–	–	–	1	
22	frag_022_C05.fsa	–	–	–	–	
23	frag_023_B05.fsa	–	1	–	1	
24	frag_024_A05.fsa	?	?	1	–	
25	frag_025_H07.fsa	–	1	–	1	
26	frag_026_G07.fsa	–	–	–	1	
27	frag_027_F07.fsa	–	1	–	1	
28	frag_028_E07.fsa	–	–	–	1	

Trace Comparison

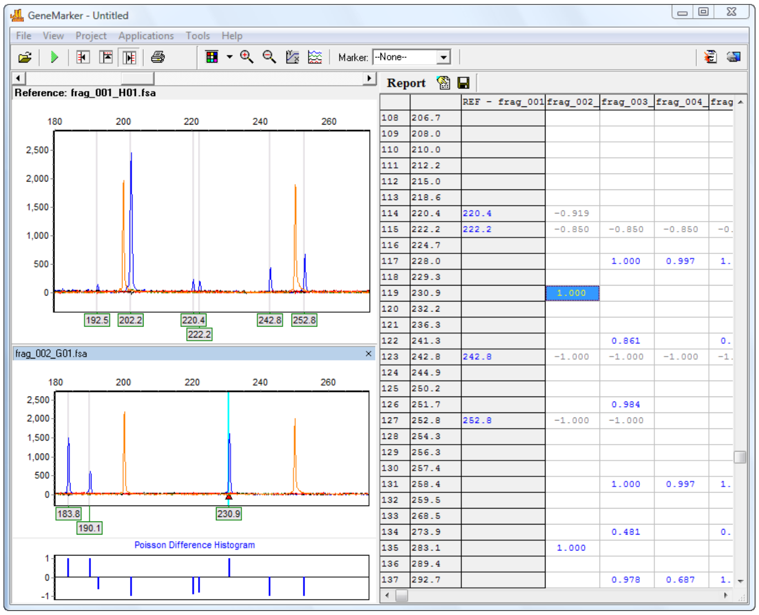
The *Trace Comparison* function for AFLP data is designed to identify length polymorphisms between closely-related species. *Trace Comparison* uses Poisson distribution to calculate significant allelic differences between samples or closely-related species. GeneMarker calculates the allelic differences between a user-defined reference and sample traces; the differences are displayed in a histogram located below each sample electropherogram. To analyze the data with the *Trace Comparison* function, it is not necessary to apply a Panel.

Overview

After the data is uploaded and filtered with Run Wizard, select *Applications* → *Trace Comparison* from the main menu bar. A new folder labeled *Trace Compare* will appear in the *Main Analysis* window *Sample List*. When samples are selected in the *Trace Compare* folder, the *Main Analysis* window will change to *Trace Comparison* mode. Select a Reference sample from the *Reference* drop-down menu in the main toolbar. The Reference sample will appear as the top *Electropherogram* and all other samples selected in the *Trace Compare* folder will be compared to that Reference sample.

The *Poisson Difference Histogram* appears below each selected sample's *Electropherogram*. A positive bar in the histogram represents a peak gain at the position as compared to the Reference and a negative bar indicates the absence of a peak at the position as compared to the Reference.

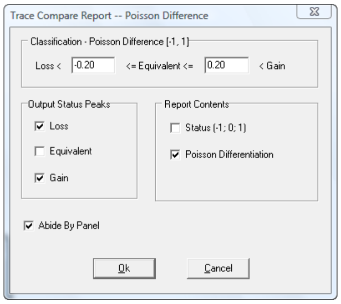
AFLP Trace Comparison



		REF - frag_001	frag_002_	frag_003_	frag_004_	frag
108	206.7					
109	208.0					
110	210.0					
111	212.2					
112	215.0					
113	218.6					
114	220.4	220.4	-0.919			
115	222.2	222.2	-0.850	-0.850	-0.850	-0.
116	224.7					
117	228.0			1.000	0.997	1.
118	229.3					
119	230.9		1.000			
120	232.2					
121	236.3					
122	241.3			0.861		0.
123	242.8	242.8	-1.000	-1.000	-1.000	-1.
124	244.9					
125	250.2					
126	251.7			0.984		
127	252.8	252.8	-1.000	-1.000		
128	254.3					
129	256.3					
130	257.4					
131	258.4			1.000	0.997	1.
132	259.5					
133	268.5					
134	273.9			0.481		0.
135	283.1		1.000			
136	289.4					
137	292.7			0.978	0.687	1.

Report Table

The Poisson Difference values are shown in the *Report Table*. Peak gains are displayed in dye-colored font and are positive numbers. Peak losses or absence are displayed in grey font and are negative values. The Poisson Difference classification for loss and gain can be modified in the *Report Settings* dialog box. To access the *Report Settings* dialog box, click the **Report Settings** icon above the *Report Table*.



Trace Compare Report -- Poisson Difference

Classification - Poisson Difference [-1, 1]

Loss < -0.20 <= Equivalent <= 0.20 < Gain

Output Status Peaks
- ☑ Loss
- ☐ Equivalent
- ☑ Gain

Report Contents
- ☐ Status (-1; 0; 1)
- ☑ Poisson Differentiation

☑ Abide By Panel

Classification – Poisson Difference

Set the Poisson Difference values at which a peak will be considered a Loss or a Gain.
Default = Loss < - *0.20* < Equivalent < *0.20* < Gain

Output Status Peaks

Choose to display *Loss*, *Equivalent*, or *Gain* values in the *Report Table*.

Report Contents

When *Status* is selected, then only -1, 0, 1 representing Loss, Equivalent, and Gain respectively will be displayed in the *Report Table*. When *Poisson Differentiation* is selected, then the Poisson Difference values will be displayed in the *Report Table*.

Abide by Panel
When selected and when a Panel has been applied to the data, only peak positions that lie within the Marker range will be reported.

Terminal-Restriction Fragment Length Polymorphism (T-RFLP)

Terminal-Restriction Fragment Length Polymorphism (T-RFLP) is a polymerase chain reaction (PCR) based genetic fingerprinting technique for the study of microbial community structure. First, DNA is extracted from a microbial community. One of the primers of a primer pair is labeled with fluorescent dye and used to amplify a selected region of a gene of interest by PCR. The resulting PCR fragment is digested with one (sometimes two or more) restriction endonuclease and the Terminal Restriction Fragments (T-RFs) are separated with an automated DNA analyzer. Microbial diversity in a community can be estimated by analyzing the number and peak heights of T-RF patterns. The aims and data analysis of T-RFLP data is similar, with minor adjustments, to the data analysis methods for AFLP.

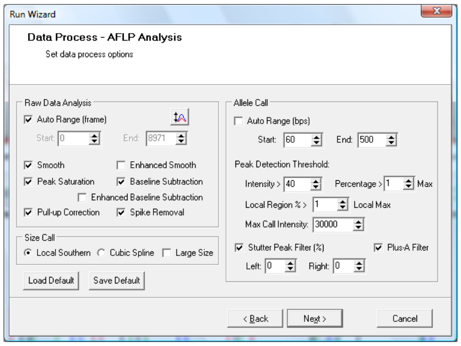
Overview

GeneMarker software has the ability to produce highly sensitive and reproducible results for T-RFLP analysis by adjusting the *AFLP Analysis* default settings. In the Run Wizard *Template Selection* box, be sure to choose the correct Size Standard, and set the *Analysis Type* as **AFLP**.

Procedure

The *Run Wizard Data Process* box has many options for auto correction and peak detection thresholds. Adjust the AFLP analysis parameters as follows:

Raw Data Analysis
    Select Smooth
Allele Call
    Deselect Auto Range
    Set Start to 60
    Set End to 500
Peak Detection Threshold
    Intensity > 40
Stutter Peak Filter
    Deselect or
    Set Left to 0%
    Set Right to 0%


Run Wizard
Data Process - AFLP Analysis
Set data process options

Raw Data Analysis
☑ Auto Range (frame)
Start: 0 End: 8971
☑ Smooth ☐ Enhanced Smooth
☑ Peak Saturation ☑ Baseline Subtraction
☐ Enhanced Baseline Subtraction
☑ Pull-up Correction ☑ Spike Removal

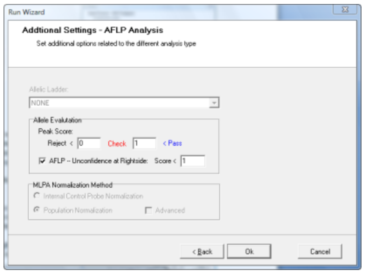
Size Call
● Local Southern ○ Cubic Spline ☐ Large Size

Allele Call
☐ Auto Range (bps)
Start: 60 End: 500
Peak Detection Threshold:
Intensity > 40 Percentage > 1 Max
Local Region % > 1 Local Max
Max Call Intensity: 30000
☑ Stutter Peak Filter (%) ☑ Plus-A Filter
Left: 0 Right: 0

NOTE: To detect low intensity peaks, T-RFLP analyses should include a low peak detection threshold with stutter filter turned off or set to zero. The peak detection threshold intensity value may be estimated based on the peak with the maximum intensity from the negative control in the sample set. As always, these parameters can easily be adjusted to optimize the analysis for your specific requirements.

The *Run Wizard Additional Settings* box displays the scoring threshold for allele peak detection.
Reject < 0 Check 1 < Pass
NOTE: If the user chooses all allele peaks with a score below 1 to be rejected, there is the possibility of a false negative, whereas if scores below 0 are rejected, there is the possibility of a false positive.

AFLP Unconfidence at Rightside Score is **1**.
NOTE: This requires that two peaks to the right of the examined peak must have a score of at least 1 for the peak to be accepted.


Run Wizard
Addtional Settings - AFLP Analysis
Set additional options related to the different analysis type

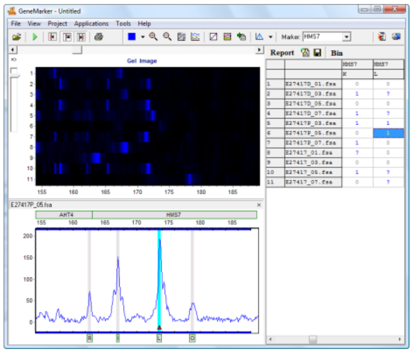
Allelic Ladder: NONE

Allele Evalutation
Peak Score:
Reject < 0 Check 1 < Pass
☑ AFLP -- Unconfidence at Rightside: Score < 1

MLPA Normalization Method
○ Internal Control Probe Normalization
● Population Normalization ☐ Advanced

What to Expect

Following size calling, the *Main Analysis* window will activate. The samples listed on the far left are marked with green symbols, indicating that size calling was successful. Sample *Electropherograms* are displayed in the center of the window. The *Report Table* on the right records the presence (1) or absence (0) of a fragment at specific positions for each sample.



Multiplex Ligation-dependent Probe Amplification (MLPA)

GeneMarker can analyze data from Multiplex Ligation-dependent Probe Amplification (MLPA). MLPA was developed by the Microbiology Research Center (MRC)-Holland in January 2002 and is a technique used to detect exon deletions in the genes for BRCA1 (associated with breast cancer), MSH2 (associated with colon cancer), and MLH1 (associated with colon cancer). MLPA can also be used to detect aneuploidy, such as Trisomy 21 found in Down syndrome.

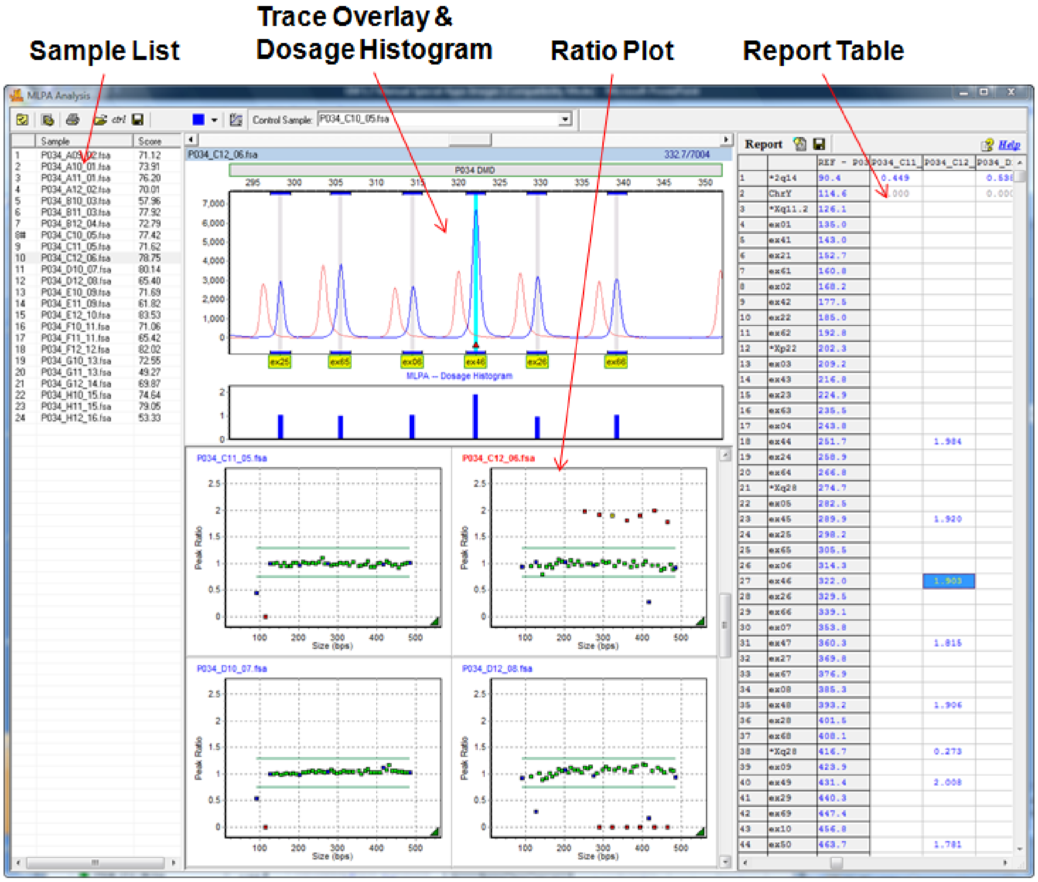
MLPA is a simple method for simultaneous quantification of up to 45 nucleic acid sequences in a single reaction. With GeneMarker, MLPA data analysis is an efficient, quick and easy process, with features such as customized reporting and printing.

In addition to the standard MLPA probe labeling technique, Luminex® Corporation has developed a method to uniquely label individual probes with different color beads. GeneMarker software can quickly and accurately analyze data from the Luminex flow cytometry instruments (Luminex 100 IS and Luminex 200) for microsphere detection.

Overview

In the MLPA Analysis window, duplications and deletions can be detected by four ways: increased or decreased peak height in the *Trace Overlay* where the reference trace appears in light red behind the dye-colored sample trace, in the *Dosage Histogram* where the histogram bar appears larger for duplications and smaller or absent for deletions, in the *Ratio Plot* where plot points represent the peak height or area ratio of the reference versus the sample, or in the *Report Table* where peak ratio or simple loss/gain information can be displayed. All frames in the MLPA Analysis window are interlinked; for example, clicking on a cell in the *Report Table* will highlight the sample in the *Sample List*, the peak in the *Overlay Trace*, and the plot point in the *Ratio Plot*.

MLPA Analysis



Sample List

Trace Overlay & Dosage Histogram

Ratio Plot

Report Table

	Sample	Score
1	P034_A09_02.fsa	71.12
2	P034_A10_01.fsa	73.91
3	P034_A11_01.fsa	76.20
4	P034_A12_02.fsa	70.01
5	P034_B10_03.fsa	57.96
6	P034_B11_03.fsa	77.92
7	P034_B12_04.fsa	72.79
8	P034_C10_05.fsa	77.42
9	P034_C11_05.fsa	71.62
10	P034_C12_06.fsa	78.75
11	P034_D10_07.fsa	80.14
12	P034_D12_08.fsa	65.40
13	P034_E10_09.fsa	71.69
14	P034_E11_09.fsa	61.82
15	P034_E12_10.fsa	83.53
16	P034_F10_11.fsa	71.06
17	P034_F11_11.fsa	65.42
18	P034_F12_12.fsa	82.02
19	P034_G10_13.fsa	72.55
20	P034_G11_13.fsa	49.27
21	P034_G12_14.fsa	69.87
22	P034_H10_15.fsa	74.64
23	P034_H11_15.fsa	79.05
24	P034_H12_16.fsa	53.33

		REF - P03	P034_C11_	P034_C12_	P034_D
1	*2q14	90.4	0.449		0.53
2	ChrY	114.6	0.000		0.00
3	*Xq11.2	126.1			
4	ex01	135.0			
5	ex41	143.0			
6	ex21	152.7			
7	ex61	160.8			
8	ex02	168.2			
9	ex42	177.5			
10	ex22	185.0			
11	ex62	192.8			
12	*Xp22	202.3			
13	ex03	209.2			
14	ex43	216.8			
15	ex23	224.9			
16	ex63	235.5			
17	ex04	243.8			
18	ex44	251.7		1.984	
19	ex24	258.9			
20	ex64	266.8			
21	*Xq28	274.7			
22	ex05	282.5			
23	ex45	289.9		1.920	
24	ex25	298.2			
25	ex65	305.5			
26	ex06	314.3			
27	ex46	322.0		1.903	
28	ex26	329.5			
29	ex66	339.1			
30	ex07	353.8			
31	ex47	360.3		1.815	
32	ex27	369.8			
33	ex67	376.9			
34	ex08	385.3			
35	ex48	393.2		1.906	
36	ex28	401.5			
37	ex68	408.1			
38	*Xq28	416.7		0.273	
39	ex09	423.9			
40	ex49	431.4		2.008	
41	ex29	440.3			
42	ex69	447.4			
43	ex10	456.8			
44	ex50	463.7		1.781	

Sample List

The *Sample List* includes the filenames of all the samples in the dataset and their respective *MLPA Score*. The *MLPA Score* is the signal-to-noise ratio that corresponds to the quality of the trace. The larger the number is, the higher the quality of the lane. Additionally, a number sign "#" appears before the sample with the least variation that was automatically selected as the *Control Sample*. This sample will also appear in the *Control Sample* drop-down menu in the toolbar. See the *What to Expect* section below for information regarding control samples and synthetic control creation.

Single left-click a sample in the *Sample List* to display the sample's *Overlay Trace* and *Ratio Plot*. Use the **Up/Down Arrow** keys to scroll through the sample list. Right-click and select *Hide* to disable a sample. Disabled samples cannot be used in the *Synthetic Control* calculation and will not appear in the *MLPA Clinical Report*. Right-click and select *Show* to enable a sample.

Trace Overlay and Dosage Histogram

The *Trace Overlay* displays a normalized electropherogram trace of the selected sample with the control sample reference trace displayed in light red behind it. The allele or exon names are displayed below their corresponding bins. See the *What to Expect* section below for information regarding trace normalization.

The *Dosage Histogram* displays, in bar chart form, the ratio of normalized peak intensities between the reference and the sample trace. Sample probes are represented by blue bars and control probes are black bars.

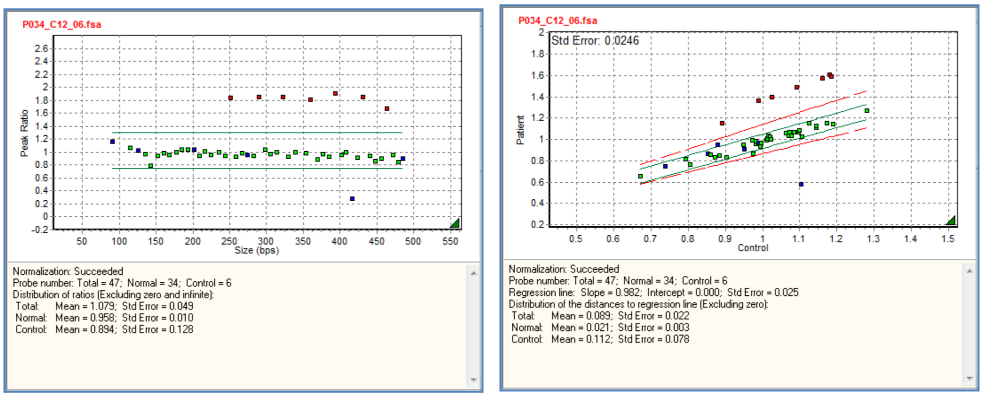
Allele editing options in the *Trace Overlay* frame are similar to those in the *Main Analysis* window *Electropherogram*. **See Chapter 3 Main Analysis Overview**.

Ratio Plots

The *Ratio Plot* displays, in graphical form, the ratio of normalized peak intensities between the reference and the sample trace. Each point represents a sample peak or probe. Select *Ratio* or *Regression Analysis* in the *MLPA Analysis Settings* box to change graphical views. Additionally, statistical information is displayed below the *Ratio Plot* when *Show Statistics Information* is selected in the *Display Settings* box.

Green = Peak is within the specified ratio limits	*Blue* = Peak is a control probe
Red = Peak is outside the specified ratio limits	*Yellow* = Current selected probe

Ratio Plot vs. Regression Plot



```
Normalization: Succeeded
Probe number: Total = 47;  Normal = 34;  Control = 6
Distribution of ratios (Excluding zero and infinite):
 Total:     Mean = 1.079;  Std Error = 0.049
 Normal:    Mean = 0.958;  Std Error = 0.010
 Control:   Mean = 0.894;  Std Error = 0.128
```

```
Normalization: Succeeded
Probe number: Total = 47;  Normal = 34;  Control = 6
Regression line:  Slope = 0.982;  Intercept = 0.000;  Std Error = 0.025
Distribution of the distances to regression line (Excluding zero):
 Total:     Mean = 0.089;  Std Error = 0.022
 Normal:    Mean = 0.021;  Std Error = 0.003
 Control:   Mean = 0.112;  Std Error = 0.078
```

Double-click a point in the *Ratio Plot* to view the peak in the *Trace Overlay* and the ratio value in the *Report Table*.

Right-click in the *Ratio Plot* and select *Adjust by Control Probes* to fit the data by only the control probes (blue points) in the sample. Right-click and select *Reset* to restore population fit calculation (all control and sample probes in the sample are used to fit the data).

If the square representing a bin is not color coded (empty) this indicates that there was no data fragment in the sample. Open square data points are the result of either a mis-aligned panel during the initial analysis or mutation specific probes that have a value of 0 RFU for normal samples. If the cause is due to mis-alignment of the panel, return to the main analysis screen, select Tools – Panel Editor and calibrate the panel (Chapter 6). If the chemistry has mutation specific probes (for example Tay Sachs and Parkinsons), the empty square will be displayed at the origin (indicating no peaks detected) in the peak ratio plots of the analysis screen and the final reports in cases where both control and sample files are normal (peak ratio will be reported as -1 (0/0 is undefined).

Report Table

The *Report Table* lists in columns the probe or exon name (according to the panel selected), the basepair size of that probe peak in the reference sample, and the ratio for each sample probe (outside the ratio limits). Double-click a cell in the *Report Table* to display the corresponding peak in the *Trace Overlay* and the plot point in the *Ratio Plot*. See the *Reports and Printing* section below for additional options in the *Report Table*.

Procedure

Analysis procedures for MLPA analysis include uploading the data, sizing, filtering and normalizing the data with Run Wizard, creating or modifying a Panel, and, finally, detecting probe insertions/deletions in the MLPA Analysis module. Below is a description of MLPA settings in Run Wizard and modifying pre-defined MRC Holland Panels in the Panel Editor. Subsequent analysis in the MLPA module will be discussed in the *What to Expect* section.

MLPA Run Wizard Settings

For explanation of specific functions in Run Wizard, **see Chapter 2 General Procedure**.

Run Wizard Template Selection

Panel: NONE OR Click the **Open Files** icon next to the *Panel* field and import the correct MLPA panel for analysis.

NOTE: MRC-Holland MLPA panels are stored in C:\ProgramFiles\SoftGenetics\GeneMarker\1.7\MLPA_Panel folder.

Size Standard: User-defined

Analysis Type: MLPA

Run Wizard Data Process

Peak Detection Intensity Threshold: 100

Global Percentage: 1% Max

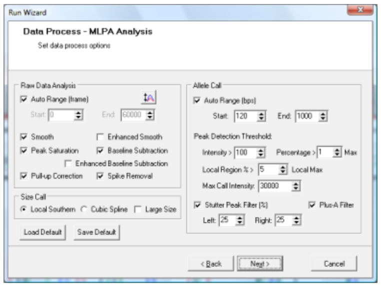
Local Percentage: 5%

Max Call Intensity: 30,000

Stutter Peak Filter: Left 25%, Right 25%

Plus A Filter: Selected

NOTE: The correct settings for MLPA Analysis should be a small global percentage plus a slightly larger or equal local percentage.

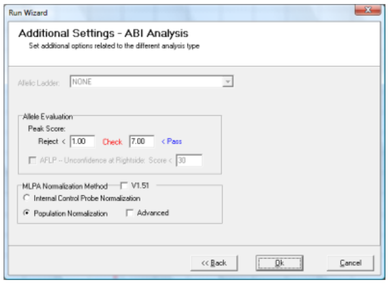

Run Wizard
Data Process - MLPA Analysis
Set data process options
Raw Data Analysis
☑ Auto Range (frame)
Start: 0 End: 60000
☑ Smooth ☐ Enhanced Smooth
☑ Peak Saturation ☑ Baseline Subtraction
☐ Enhanced Baseline Subtraction
☑ Pull-up Correction ☑ Spike Removal
Size Call
● Local Southern ○ Cubic Spline ☐ Large Size
Allele Call
☑ Auto Range (bps)
Start: 120 End: 1000
Peak Detection Threshold:
Intensity > 100 Percentage > 1 Max
Local Region % > 5 Local Max
Max Call Intensity: 30000
☑ Stutter Peak Filter (%) ☑ Plus-A Filter
Left: 25 Right: 25

Run Wizard Additional Settings

Peak Score Allele Evaluation

Reject < 1 Check < 10 < Pass

NOTE: If the user chooses all allele peaks with a score below 1 to be rejected, there is the possibility of a false negative, whereas if scores below 0 are rejected, there is the possibility of a false positive.


Run Wizard
Additional Settings - ABI Analysis
Set additional options related to the different analysis type
Allelic Ladder: NONE
Allele Evaluation
Peak Score:
Reject < 1.00 Check 7.00 < Pass
☐ AFLP -- Unconfidence at Rightside: Score < 30
MLPA Normalization Method ☐ V1.51
○ Internal Control Probe Normalization
● Population Normalization ☐ Advanced

MLPA Normalization Method

Normalization adjusts the peak intensities within individual samples to the same scale for comparative purposes.

Internal Probe Normalization: Adjusts peak intensities based on the intensities of the probes designated as controls in the *Panel Editor*.

<u>*Population Normalization*</u>: Adjusts peak intensities based on an average of peak intensities from all probes of high quality samples.
NOTE: *Population Normalization* is the recommended method for MLPA Analysis.

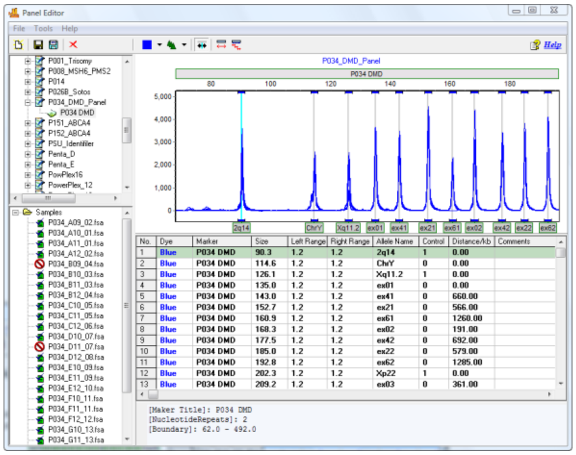
<u>*Advanced*</u>: Available when *Population Normalization* method is selected. Choose *Advanced* when samples contain deletion or duplications in more than half of the peaks (probes).

<u>*V1.51*</u> This option uses the normalization algorithm from GeneMarker version 1.51

MLPA Panel Selection and Modification

After the data has been sized, filtered, and normalized with Run Wizard, go to *Tools → Panel Editor* to adjust the MRC-Holland Panel selected. To create a new Panel or for explanation of specific functions in *Panel Editor*, **see Chapter 5 Panel Editor**.

1. In *Panel Editor*, select the appropriate MRC-Holland Panel for the dataset from the *Panel List*.
   a. If the Panel does not appear in the *Panel List*, go to *File → Import Pre-Defined Panels.*
   b. The C:\Program Files\SoftGenetics\GeneMarker\1.7\MLPA_Panel folder will appear.
   c. Select the correct Panel and click **Open**.
   d. The Panel will now appear in the Panel List.
   NOTE: Information regarding MRC-Holland Panels can be located on their website at www.mrc-holland.com



No.	Dye	Marker	Size	Left Range	Right Range	Allele Name	Control	Distance/kb	Comments
1	Blue	P034 DMD	90.3	1.2	1.2	2q14	1	0.00	
2	Blue	P034 DMD	114.6	1.2	1.2	ChrY	0	0.00	
3	Blue	P034 DMD	126.1	1.2	1.2	Xq11.2	1	0.00	
4	Blue	P034 DMD	135.0	1.2	1.2	ex01	0	0.00	
5	Blue	P034 DMD	143.0	1.2	1.2	ex41	0	660.00	
6	Blue	P034 DMD	152.7	1.2	1.2	ex21	0	566.00	
7	Blue	P034 DMD	160.9	1.2	1.2	ex61	0	1260.00	
8	Blue	P034 DMD	168.3	1.2	1.2	ex02	0	191.00	
9	Blue	P034 DMD	177.5	1.2	1.2	ex42	0	692.00	
10	Blue	P034 DMD	185.0	1.2	1.2	ex22	0	579.00	
11	Blue	P034 DMD	192.8	1.2	1.2	ex62	0	1285.00	
12	Blue	P034 DMD	202.3	1.2	1.2	Xp22	1	0.00	
13	Blue	P034 DMD	209.2	1.2	1.2	ex03	0	361.00	

```
[Maker Title]: P034 DMD
[NucleotideRepeats]: 2
[Boundary]: 62.0 - 492.0
```

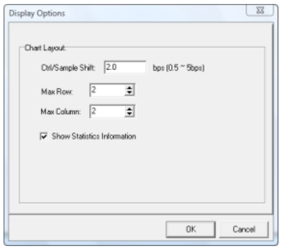
2. Adjust the Markers and Bins to align with sample peaks.
3. Identify the Control Probe peaks
   a. Right-click a Bin in the *Overlay Trace* or *Panel Table*. Select *Edit Allele*. Click the **Control Gene** checkbox in the *Allele Editor* dialog box. Click **OK.**
   <u>*OR*</u>
   b. Enter a "1" in the *Control* column of the *Panel Table* and enter a -1 for quality control fragments
   <u>*OR*</u>
   c. Right-click a Bin in the *Overlay Trace* or *Panel Table* and select *Set as Control/ Set as Non-control*
4. After editing, select *File → Save Changes* (**Hot Key = CTRL+S**) <u>*OR*</u> click the **Save Changes** icon
5. Exit the *Panel Editor*
6. Click the **Run Process** icon in the *Main Analysis* window
7. Select the adjusted Panel from the Run Wizard *Template Selection Panel* drop-down menu
8. Proceed through Run Wizard and click **OK** in the *Data Process* box
9. The Panel has been applied

Launch MLPA Analysis Module

10. In the main toolbar, select *Applications → MLPA Analysis*
11. The *MLPA Analysis Settings* dialog window will activate
12. Choose the appropriate analysis parameters
13. Click **OK**
14. The *MLPA Analysis* window will appear

Icons and Functions

Display Settings: Allows the user to select the preferred chart layout display. A maximum of 5 rows and 5 columns is permitted. Statistical information will be included under each chart when the *Show Statistics*



Ctrl/Sample Shift: 2.0 bps (0.5 ~ 5bps)
Max Row: 2
Max Column: 2
☑ Show Statistics Information

Information option is selected.

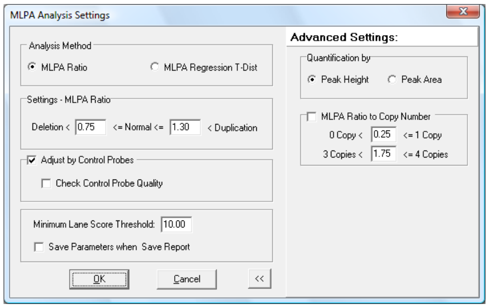
MLPA Analysis Settings
Launches the *MLPA Analysis Settings* box

MLPA Ratio

MLPA Ratio is a simple and easy method for detecting duplications and deletions. The sample peak heights or areas are compared to a control/reference sample and the ratio value is used to determine the addition or absence of the allele probe.

Settings – MLPA Ratio: Allows the user to input the deletion and duplication values for the analysis. The default setting is Deletion < **0.75** < Normal < **1.30** < Duplication.

Adjust by Control Probes: When selected, all samples with control probes designated in the panel will be adjusted by that sample's control probes. The method of adjustment can be changed in each individual sample by right-clicking in the *Ratio Plot* of the sample and selecting **Adjust by Control Probes** or **Reset**.



MLPA Analysis Settings

Analysis Method
● MLPA Ratio ○ MLPA Regression T-Dist

Settings - MLPA Ratio
Deletion < 0.75 <= Normal <= 1.30 < Duplication

☑ Adjust by Control Probes
☐ Check Control Probe Quality

Minimum Lane Score Threshold: 10.00
☐ Save Parameters when Save Report

Advanced Settings:

Quantification by
● Peak Height ○ Peak Area

☐ MLPA Ratio to Copy Number
0 Copy < 0.25 <= 1 Copy
3 Copies < 1.75 <= 4 Copies

Save Parameters when Save Report: Automatically generates an *.ini file of the MLPA Analysis Parameters when the MLPA report is saved. Naming convention for the file is Report file name_MLPA_Settings.ini

Adjust by Control Probes and *Check Control Probe Quality*: When selected, all samples with control probes that meet a certain quality criteria (minimal amount of variation among control probe intensities) will be adjusted by the sample's control probes. All other samples will be fitted based on the population of control and test probes. The method of adjustment can be changed in each individual sample by right-clicking in the *Ratio Plot* of the sample and selecting **Adjust by Control Probes** or **Reset**.

Minimum Lane Score Threshold: Sample files are given a signal-to-noise type score (0 to 100) that corresponds to the quality of the trace. To view only the samples above a certain score threshold, set the *Minimum Lane Score Threshold* to the desired value. All samples below the selected value (score) will be deactivated and appear grey in the *Sample List*.

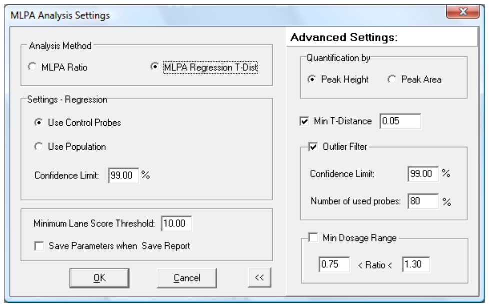
Quantification: Select whether to calculate peak ratios by area or height. To view the *Quantification* option, click the double arrow button at the bottom of the dialog box.

MLPA Ratio to Copy Number: When selected, the software will convert peak ratios to copy number values applying user defined parameters. The Normal range in 'Settings-MLPA Ratio' is defined as 2 copies, Deletion as 1 copy and Duplication as 3 copies. The copy numbers will be applied to the Allele and Patient reports. To view the *MLPA Ratio to Copy Number* option, click the double arrow button at the bottom of the dialog box.

MLPA Regression T-Distribution

Uses the T-distribution calculation to compare reference and sample peak heights or areas. Using T-distribution method requires confidence limits to be set.

Settings – Regression: Choose *Use Control Probes* to analyze each sample by the identified control probes. Choose *Use Population* to analyze each sample by all probes in the sample. The *Confidence Limit* is represented by the green lines in the *Ratio Plot* and can be used to determine which probe points are outliers as compared to all probes (population) or control probes in the sample. The default setting is **99%**.



MLPA Analysis Settings

Analysis Method
○ MLPA Ratio ● MLPA Regression T-Dist

Settings - Regression
● Use Control Probes
○ Use Population
Confidence Limit: 99.00 %

Minimum Lane Score Threshold: 10.00
☐ Save Parameters when Save Report

Advanced Settings:

Quantification by
● Peak Height ○ Peak Area

☑ Min T-Distance 0.05

☑ Outlier Filter
Confidence Limit: 99.00 %
Number of used probes: 80 %

☐ Min Dosage Range
0.75 < Ratio < 1.30

Minimum Lane Score Threshold: Sample files are given a signal-to-noise type score (0 to 100) that corresponds to the quality of the trace. To view only the samples above a certain score threshold, set the *Minimum Lane Score Threshold* to the desired value. All samples below the selected value (score) will be deactivated and appear grey in the *Sample List*. The default setting is **10**.

Save Parameters when Save Report: Automatically generates an *.ini file of the MLPA Analysis Parameters when the MLPA report is saved. Naming convention for the file is Report file name_MLPA_Settings.ini

Quantification: Select whether to calculate peak ratios by area or height. To view *Advanced Settings*, click the double arrow button at the bottom of the dialog box.

Min T-Distance: Helps to reduce false positives in high quality samples by setting the minimum distance the alleles must be from one another to be considered true peaks. The default setting is **0.05**.

Outlier Filter; Confidence Limit; Number of Probes Used: This feature allows the user to define the confidence in the calling of outliers. For example, 99% confidence means that GeneMarker is 99% sure that the alleles considered as outliers are truly deletions and duplications. The *Number of Probes Used* allows the user to set the percentage of probes used for each iteration of normalization in order to reach the desired confidence. The default is **80%**, which means that 20% of the probes will be disregarded before each regression line fit.

Min Dosage Range: The *Minimum Dosage Range* helps to reduce false positives by setting the thresholds that determine the calling of deletions and duplications to desired settings. This is shown as red lines radiating from the origin (0, 0) of the regression plot. The default setting is **Deletion < 0.75 < Normal < 1.30 < Duplication.**

Print Report
Launches the *MLPA Print Settings* box. See *Reports and Printing* section below for more information.

Load Control Sample From a Database
Individually load a control sample to be used in the analysis

Control Sample Selection
Launches the *Control Sample Selection* box which lists the dataset samples that can be selected and used as the synthetic control for MLPA analysis.

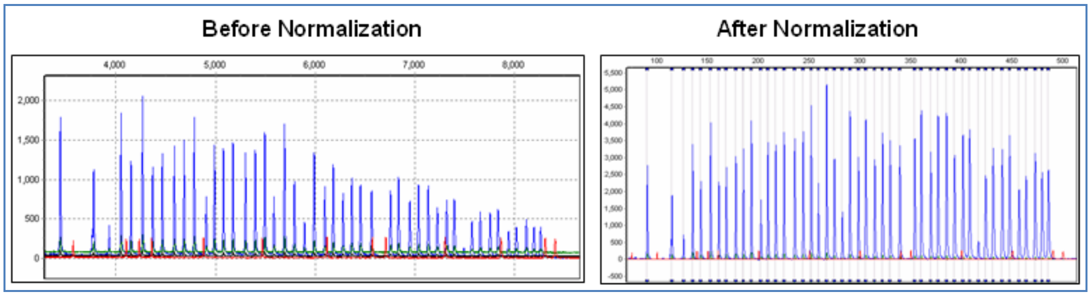
Save Synthetic Sample
Saves the synthetic control sample as a Text (.txt) or Trace (.scf or .sg1) file

What to Expect

After initial analysis with Run Wizard and adjustment of analysis settings, the sample data must be reviewed for normalization accuracy, sample lane quality, and control sample identification prior to final deletion/duplication determination.

Verify Normalization Accuracy

Normalization is the adjustment of peak heights in a sample to minimize or remove the effects of preferential amplification of smaller fragments during PCR. Notice in the before and after example below how initially the sample's peaks are increasingly shorter as the fragment size increases. After the normalization calculation is applied, the peak heights are consistent from the beginning to the end of the trace. Normalization is important for MLPA analysis because the sample's peak heights/areas are compared to a control/reference trace. If the peak heights are not consistent, then false positive deletion/duplication calls may result.



To verify normalization accuracy in the MLPA Analysis window, locate the color-coded triangle in the bottom-right corner of the *Ratio Plots*. A green triangle represents successful normalization, yellow represents normalization that needs to be verified for accuracy, and red indicates the normalization failed for that sample. If several samples failed normalization, go back to the Run Wizard and change the *Internal Control Probe* or *Population Normalization/Advanced Normalization* option in the *Additional Settings* box.

NOTE: A Panel must be applied to the dataset in order for normalization to occur.

Due to the variations of PCR efficiencies from small to large DNA fragments or from sample to sample, two selectable normalization methods are provided. GeneMarker can normalize MLPA-derived data by an internal control probe method or by a population method. In order to correct for the peak intensity variation over size, an exponential function, $a*e^{-bz}$, is used to fit to the square root of peak intensities, where **z** is size, and **a** and **b** are fitting constants. Essentially, both normalization methods take the square root of the intensity ratio and plot the ratios to model a linear regression.

Internal Control Probe Normalization[1]

The Internal Control Probe Normalization method corrects the trend of dropping intensities by setting the height ratio of the control probes to 1.00 and then calculating ratios for all other probes in the sample as compared to the controls. The control probe peaks are identified in the selected Panel with a "1" in the Control column.

NOTE: Some MLPA probe kits do not contain control probes.

Because the trend of control peak intensities varies greatly from one sample to another, the Population Normalization method is the recommended method for most datasets.

[1] Lampros A. Mavrogiannia, et al., St. James's University Hospital, Leeds.
http://leedsdna.info/science/dosage/REX-MLPA/REX-MLPA_CMGS04.ppt

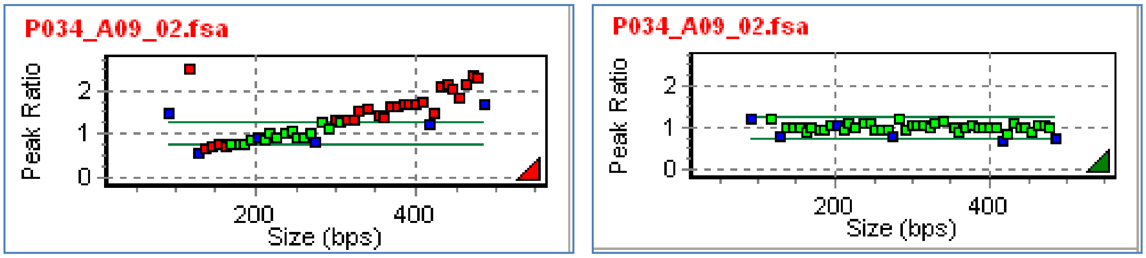
http://www.eurogentest.org/uploads/1247475007479/MLPA_validationreport_TJ_version1_20090710.pdf

Population Normalization

The Population Normalization method uses all peaks in the sample to correct for preferential amplification effects. Median peak intensities are derived from the first nine data points in a trace, then sliding to data points 2-10, 3-11, etc., to ascertain the local median intensities. This median filter reduces variation in peak intensities among the probes. All of the median intensities are then used to fit the exponential function, which results in higher accuracy and lower false positive rates.

NOTE: Because control probes can fail or may contain duplications or deletions, Population Normalization is a more robust method of normalization as compared to Internal Control Probe Normalization.

Internal Control Probe vs. Population Normalization



Advanced Population Normalization

The Advanced Population Normalization method is designed to normalize data that contains a large number of test probes displaying copy number change. Problematic data sets that are difficult to normalize using the other two methods can usually be successfully normalized using the *Advanced* function. To activate Advanced Population Normalization, select Population Normalization and check the **Advanced** option in the *Run Wizard Additional Settings* box.

Advanced Population Normalization solves problems when other normalization methods prove less than optimal. It is a combination of Population and Internal Control Probe normalization methods. Some samples that contain many probes with deletion or duplication copy number changes may be difficult to normalize. When samples display an upward trend in peak ratios it is an indication of sub-optimal normalization. The *Advanced* option uses the trend in these data to adjust the slope. This option works best if control probes have been assigned.

GeneMarker uses a cluster algorithm to differentiate well separated data points and then uses those clusters to calculate a corrected slope. There are usually two populations (clusters) of peak ratios. Initially, the peak ratio plots are divided into approximately 4 equal parts. The upper 25% peak ratios for each of the top two quadrants and the lower 25% peak ratios for each of the lower two quadrants are retained. The middle values are not used in these statistics to avoid data points that are close to the quadrant boundary. The retained values are used to calculate a slope for the upper two and the lower two quadrants. These slopes are an estimate of the trend in the data and a corrected slope is plotted.

Verify Sample Lane Quality

After normalization has been verified, check the *Sample List* in the MLPA Analysis window to identify samples that received a lane score below the *Minimum Lane Score Threshold*. Samples with low lane score will be disabled and grey out in the *Sample List*. To view the disabled sample's probes in the *Ratio Plot*, right-click on the sample in the *Sample List* and select **Show**.

Control Sample Identification

When MLPA Analysis is launched, the software automatically selects a sample in the dataset to use as the control. The **"#"** sign next to the sample number indicates the automatically selected control. While the chosen sample may not actually be the experimental control, this selection is based upon which sample presents the data with the fewest abnormal calls. The user may change the control to reflect the experimental sample or another sample which they feel portrays the data in a meaningful fashion.

To select a control sample:

Click on the **Control Sample** drop-down menu and select the desired control sample.

OR

Select the **Load Control Sample From a Database** icon. Selecting a control sample using this method requires the sample be in TEXT or SG1 file format.

Synthetic Control Sample

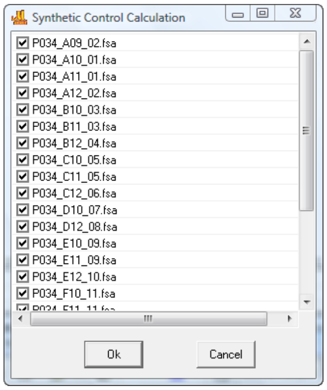
GeneMarker's *MLPA Analysis* allows users to create a synthetic control sample that can be used as a control/reference sample. This sample is calculated as the median of all selected sample files.

To select sample files to be used in the calculation for the control sample:

1. Click on the **Control Sample Selection** icon.
2. The *Synthetic Control Calculation* box appears.
3. Click the checkbox next to samples to include in the Synthetic Control.

NOTE: Sample files with a score below the *Minimum Lane Score Threshold* cannot be used in the control sample selection.

4. Click **OK**
5. The *Synthetic Control Sample* is automatically selected in the **Control Sample** drop-down menu.
6. The synthetic control sample can be saved as SCF (4-color data), SG1 (5-color data) or TXT file formats by clicking the **Save** icon.
7. To list the synthetic control samples in the MLPA Clinical Report, click the **Print Report** icon.
8. The *MLPA Print* box will appear.
9. Click the check box next to *List Samples Used for Synthetic Control*. A maximum of **50** synthetic controls can be selected.


Synthetic Control Calculation

☑ P034_A09_02.fsa
☑ P034_A10_01.fsa
☑ P034_A11_01.fsa
☑ P034_A12_02.fsa
☑ P034_B10_03.fsa
☑ P034_B11_03.fsa
☑ P034_B12_04.fsa
☑ P034_C10_05.fsa
☑ P034_C11_05.fsa
☑ P034_C12_06.fsa
☑ P034_D10_07.fsa
☑ P034_D12_08.fsa
☑ P034_E10_09.fsa
☑ P034_E11_09.fsa
☑ P034_E12_10.fsa
☑ P034_F10_11.fsa

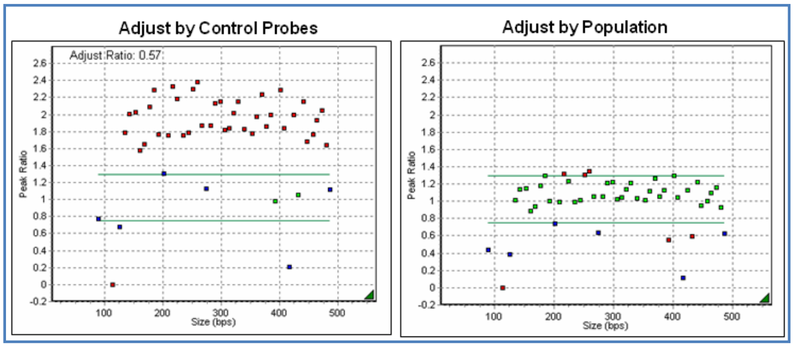
MLPA Analysis

GeneMarker features two types of MLPA analyses: MLPA Ratio and MLPA Regression. Both MLPA analysis features identify data points that are outliers. Outlying points are determined based on the deviation of each allele peak, relative to the average deviation of all peaks. Any individual peak whose residual deviation is greater than 3 times the average deviation will be considered as outlying to the data. These outlying points should be considered as possible duplications or deletions and need to be reviewed by the user.

MLPA Ratio Method

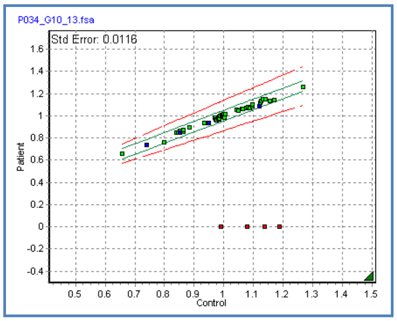
MLPA Ratio plots the ratio of each sample compared to the panel. In MLPA Ratio, the ratio of the sample data to the control sample is standardized, such that the median point within the data set is considered to be 1. All other data points are relative to the median value, as depicted in the plots. The outlying data points represent duplications and deletions. Duplications will be shown "above" the plotted ratio line, and deletions will be "below" the line. When you double-click on any point, the dosage histogram and the report will also display the selected point.

In MLPA Ratio Analysis, it is possible to toggle between the Population and Control Probe normalization by right-clicking in each sample graph. For some samples, the Control Probe normalization may be more accurate, when the control probes and the test probes are in two distinct groups.



MLPA Regression Method

MLPA Regression analysis relies upon a T-Distribution to form the regression line between the square root of the sample and the square root of the control. GeneMarker utilizes an automatic iteration-based regression method. Using this method, the software forms a best-fit line, retaining 80% of peaks with smaller deviations and 20% are rejected. GeneMarker then iterates multiple times to reject and retain peaks, with a confidence of 99%. This process is repeated until the regression line has reached the desired confidence. Finally, the removed data points are placed back into the plot and will show up either on the regression line or as outliers.



Reports and Printing

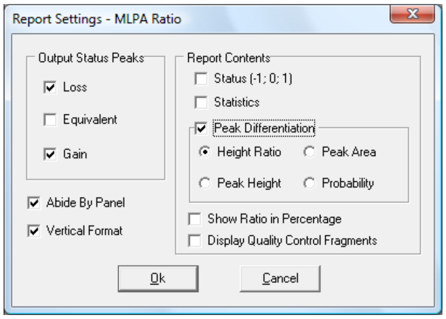
GeneMarker automates the analysis process and creates professional clinical reports for accurate insertion/deletion detection.

Report Table

Displays gain and loss information for individual peaks of a sample compared to a reference. Samples designated as "reference" by the filename group text file are marked as blue rows in the report while samples being compared to the reference are in the white rows. The *Overlay Trace* window will correspond to which ever peak cell is selected in the *Report Table*.

Report Settings

Launches the *Report Options* dialog box.


Report Settings - MLPA Ratio

Output Status Peaks
☑ Loss
☐ Equivalent
☑ Gain

☑ Abide By Panel
☑ Vertical Format

Report Contents
☐ Status (-1; 0; 1)
☐ Statistics
☑ Peak Differentiation
● Height Ratio ○ Peak Area
○ Peak Height ○ Probability
☐ Show Ratio in Percentage
☐ Display Quality Control Fragments

*Output Status Peaks***:** Selecting this option displays the peak intensity ratio values in the allele report.

*Abide by Panel***:** Displays allele information that is within the selected panel. Alleles outside of the selected panel will be represented by an "OL".

*Vertical Format***:** Arranges the allele report in a vertical format.

*Report Contents***:** Selecting Status (-1, 0, 1) will display the values of -1, 0, and 1 for each detected allele to represent the presence, absence, and questionable presence of alleles, respectively.

Statistics: Displays the number of probes, mean, standard error and standard deviation for the control and sample probes at the bottom of the MLPA ratio report table.

*Peak Differentiation***:** Options to display peak differentiation in terms of peak height ratio, peak area, peak height, and probability are available. These calculations will be applied to the peaks selected to display in the *Output Status Peaks*.

*Show Ratio in Percentage***:** Displays the peak height ratio, in percentage, if this option is selected.

Display Quality Control Fragments: Displays the quality control fragments for panels that contain quality control fragments designated with a -1 (negative 1) in the Control column in the panel editor.

Save Report

Choose to save the *Report Table* as an Excel file (.xls) or a tab-delimited Text file (.txt).

MLPA Clinical Report Settings

Click the **Print Report** icon to launch the *MLPA Print* dialog box and preview, print or save the *MLPA Clinical Report*.

Samples

Select *All Samples* or just the samples you wish to report. To *Select Samples*, click the "…" button next to Selected Samples and place a check mark in the box next to the samples you wish to appear in the report.

Sort Probe

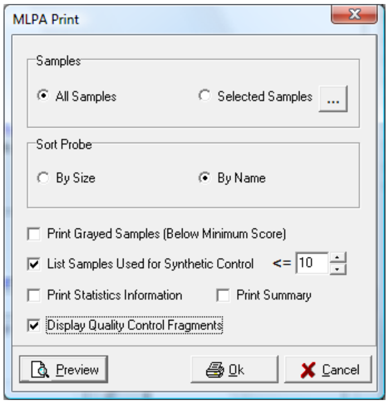
Sort the probes in descending order *By Size* or *By Name* in the report table.

Print Grayed Samples

When selected, the report will include samples below the minimum score set in the *MLPA Settings* box (*Minimum Lane Score Threshold*).

List Samples Used for Synthetic Control

When selected, a maximum of ten samples will be displayed in the printed report. If more than ten samples are used, the symbol "…" will be displayed after the tenth sample name.


MLPA Print

Samples
● All Samples ○ Selected Samples

Sort Probe
○ By Size ● By Name

☐ Print Grayed Samples (Below Minimum Score)
☑ List Samples Used for Synthetic Control <= 10
☐ Print Statistics Information ☐ Print Summary
☑ Display Quality Control Fragments

Print Statistics Information

Displays the number of probes, mean and standard deviation for the reference and sample in the bottom left corner of the report.

Print Summary

Attaches an extra report to the end of the Clinical Reports that summarizes the ratio information for all the selected samples.

Display Quality Control Fragments

Displays the quality control fragments in the print report

MLPA Clinical Research Report

Below is a description of the *MLPA Clinical Report* features. For an explanation of functions within the *Print Preview* window, see **Chapter 6 Reports and Printing**. If there are more than 50 probes in the analysis a second page is generated with the remaining alleles in a table, the header, trace overlay, conclusion box and ratio plot.

Report Header

Contains information about the analysis, project, sample and parameters.

Overlay Trace

Similar to the MLPA Analysis window, displays the reference trace in light red behind the sample trace (dye color). The zoom setting in the MLPA Analysis window is applied in the Print Report.

Ratio Plot

Similar to the MLPA Analysis window, the plot points indicate the Peak Height/Area Ratio of sample to reference.

Statistics Information

When selected, the statistics information is displayed above the Signature box and contains sample information that includes number of control/sample probes, mean peak ratio for control/sample probes, and the standard deviation of the probes.

Signature Box

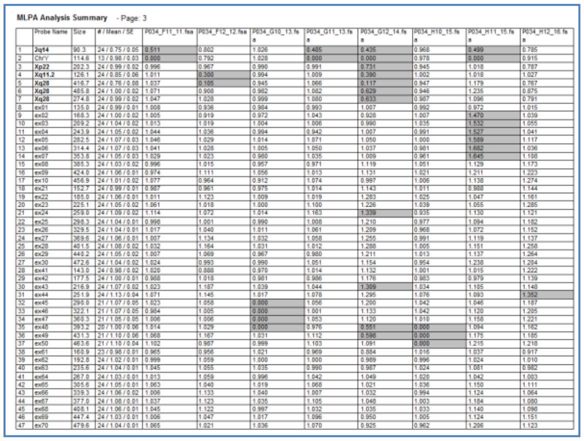
Date and initial space for report reviewers.

Duplication/Deletion Report Table

A list of all probes in the samples, fragment basepair size, and peak ratio information. Peak ratio cells which meet the duplication/deletion threshold criteria are highlighted grey.

Summary Report

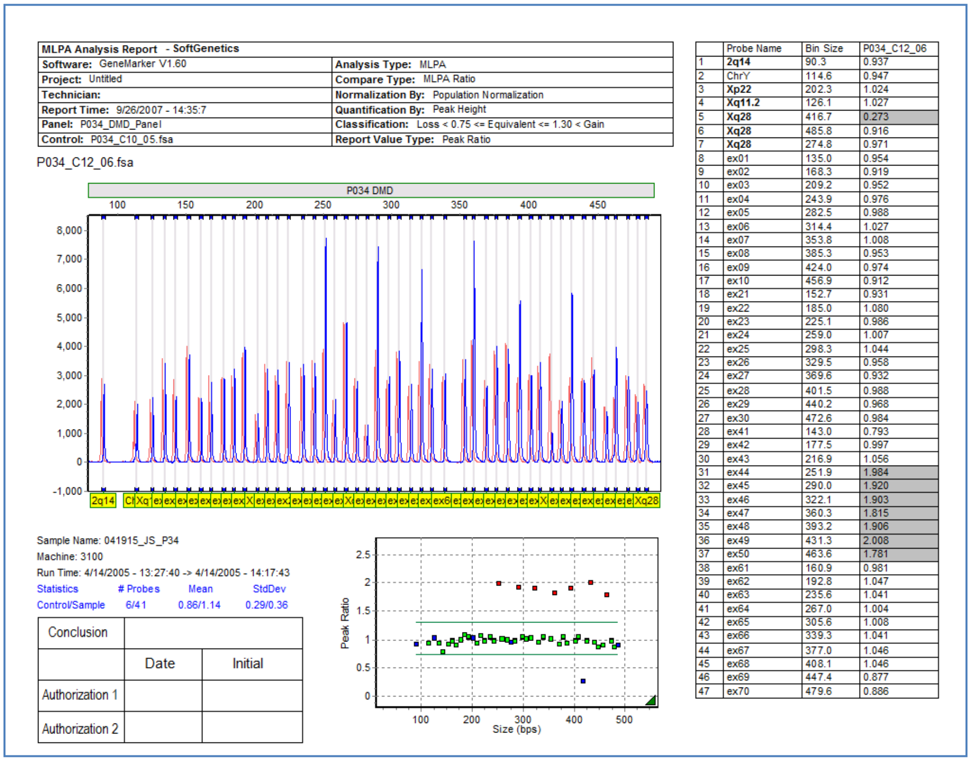
When *Print Summary* is selected in the *MLPA Print Settings* box, additional print report sheets appear after all the sample sheets. The *Summary Report* lists all the probes in the dataset and reports peak ratio information in sample columns. Peak ratio cells which meet the insertion/deletion threshold criteria are highlighted grey. MLPA Clinical Report



MLPA Analysis Summary - Page: 3

	Probe Name	Size	# / Mean / SE	P034_F11_11.fsa	P034_F12_12.fsa	P034_G10_13.fsa	P034_G11_13.fsa	P034_G12_14.fsa	P034_H10_15.fsa	P034_H11_15.fsa	P034_H12_16.fsa
1	**2q14**	90.3	24 / 0.75 / 0.05	0.511	0.802	1.026	0.485	0.435	0.968	0.499	0.785
2	ChrY	114.6	13 / 0.98 / 0.03	0.000	0.792	1.028	0.000	0.000	0.978	0.000	0.915
3	**Xp22**	202.3	24 / 0.99 / 0.02	0.996	0.967	0.990	0.991	0.731	0.945	1.018	0.787
4	**Xq11.2**	126.1	24 / 0.85 / 0.06	1.011	0.300	0.994	1.009	0.390	1.002	1.018	1.027
5	**Xq28**	416.7	24 / 0.76 / 0.08	1.037	0.105	0.945	1.066	0.117	0.947	1.179	0.767
6	**Xq28**	485.8	24 / 1.00 / 0.02	1.071	0.908	0.982	1.082	0.629	0.946	1.235	0.875
7	**Xq28**	274.8	24 / 0.99 / 0.02	1.047	1.028	0.999	1.080	0.633	0.987	1.096	0.791
8	ex01	135.0	24 / 0.99 / 0.01	1.008	0.936	0.984	0.993	1.007	0.992	0.972	1.015
9	ex02	168.3	24 / 1.00 / 0.02	1.005	0.919	0.972	1.043	0.928	1.007	1.470	1.039
10	ex03	209.2	24 / 1.04 / 0.02	1.013	1.019	1.004	1.006	0.990	1.035	1.532	1.055
11	ex04	243.9	24 / 1.05 / 0.02	1.044	1.036	0.994	0.942	1.007	0.991	1.527	1.041
12	ex05	282.5	24 / 1.07 / 0.03	1.046	1.029	1.014	1.071	1.050	1.000	1.589	1.117
13	ex06	314.4	24 / 1.07 / 0.03	1.041	1.028	1.005	1.050	1.037	0.981	1.682	1.036
14	ex07	353.8	24 / 1.05 / 0.03	1.029	1.023	0.980	1.035	1.009	0.961	1.645	1.108
15	ex08	385.3	24 / 1.03 / 0.02	0.996	1.015	0.957	0.971	1.119	1.051	1.129	1.173
16	ex09	424.0	24 / 1.06 / 0.01	0.974	1.111	1.056	1.013	1.131	1.021	1.211	1.223
17	ex10	456.9	24 / 1.01 / 0.02	1.077	0.964	0.912	1.074	0.997	1.006	1.138	1.274
18	ex21	152.7	24 / 0.99 / 0.01	0.987	0.961	0.975	1.014	1.143	1.011	0.988	1.144
19	ex22	185.0	24 / 1.06 / 0.01	1.011	1.123	1.009	1.019	1.283	1.025	1.047	1.161
20	ex23	225.1	24 / 1.05 / 0.02	1.061	1.018	1.000	1.100	1.226	1.039	1.055	1.285
21	ex24	259.0	24 / 1.09 / 0.02	1.114	1.072	1.014	1.163	1.339	0.935	1.130	1.121
22	ex25	298.3	24 / 1.04 / 0.01	0.998	1.001	0.990	1.008	1.210	0.977	1.094	1.182
23	ex26	329.5	24 / 1.04 / 0.01	1.017	1.040	1.011	1.061	1.209	0.968	1.072	1.152
24	ex27	369.6	24 / 1.06 / 0.01	1.007	1.134	1.032	1.058	1.255	0.991	1.119	1.137
25	ex28	401.5	24 / 1.08 / 0.02	1.032	1.164	1.031	1.012	1.288	1.005	1.151	1.258
26	ex29	440.2	24 / 1.05 / 0.02	1.007	1.069	0.967	0.980	1.211	1.013	1.137	1.264
27	ex30	472.6	24 / 1.04 / 0.02	1.024	0.993	0.990	1.051	1.154	0.954	1.238	1.204
28	ex41	143.0	24 / 0.98 / 0.02	1.020	0.888	0.970	1.014	1.132	1.001	1.015	1.222
29	ex42	177.5	24 / 1.00 / 0.01	0.988	1.018	0.981	0.986	1.176	0.983	0.979	1.139
30	ex43	216.9	24 / 1.07 / 0.02	1.023	1.187	1.039	1.044	1.309	1.034	1.105	1.148
31	ex44	251.9	24 / 1.13 / 0.04	1.071	1.145	1.017	1.078	1.295	1.076	1.093	1.352
32	ex45	290.0	21 / 1.07 / 0.05	1.023	1.058	0.000	1.056	1.200	1.042	1.046	1.187
33	ex46	322.1	21 / 1.07 / 0.05	0.984	1.005	0.000	1.001	1.133	1.042	1.120	1.205
34	ex47	360.3	21 / 1.05 / 0.05	1.006	1.006	0.000	1.053	1.120	1.010	1.158	1.221
35	ex48	393.2	20 / 1.00 / 0.06	1.014	1.029	0.000	0.976	0.551	0.000	1.094	1.162
36	ex49	431.3	21 / 1.10 / 0.06	1.068	1.167	1.031	1.112	0.598	0.000	1.175	1.185
37	ex50	463.6	21 / 1.10 / 0.04	1.102	0.987	0.999	1.103	1.091	0.000	1.215	1.218
38	ex61	160.9	23 / 0.98 / 0.01	0.965	0.956	1.021	0.969	0.884	1.016	1.037	0.917
39	ex62	192.8	24 / 1.02 / 0.01	0.999	1.059	1.000	1.000	0.989	0.996	1.024	1.010
40	ex63	235.6	24 / 1.04 / 0.01	1.045	1.055	1.035	0.990	0.987	1.024	1.081	0.982
41	ex64	267.0	24 / 1.03 / 0.01	1.013	1.059	0.996	1.042	1.049	1.020	1.042	1.003
42	ex65	305.6	24 / 1.05 / 0.01	1.063	1.040	1.019	1.068	1.021	1.036	1.150	1.111
43	ex66	339.3	24 / 1.06 / 0.02	1.006	1.133	1.040	1.007	1.032	0.994	1.124	1.064
44	ex67	377.0	24 / 1.08 / 0.01	1.037	1.123	1.035	1.105	1.048	1.003	1.184	1.080
45	ex68	408.1	24 / 1.06 / 0.01	1.045	1.122	0.997	1.032	1.035	1.033	1.140	1.098
46	ex69	447.4	24 / 1.03 / 0.01	1.006	1.047	1.017	1.096	0.950	1.005	1.124	1.151
47	ex70	479.6	24 / 1.04 / 0.01	1.065	1.021	1.036	1.070	0.925	0.962	1.206	1.123

Final MLPA Report



MLPA Analysis Report - SoftGenetics	
Software: GeneMarker V1.60	**Analysis Type:** MLPA
Project: Untitled	**Compare Type:** MLPA Ratio
Technician:	**Normalization By:** Population Normalization
Report Time: 9/26/2007 - 14:35:7	**Quantification By:** Peak Height
Panel: P034_DMD_Panel	**Classification:** Loss < 0.75 <= Equivalent <= 1.30 < Gain
Control: P034_C10_05.fsa	**Report Value Type:** Peak Ratio

P034_C12_06.fsa

Sample Name: 041915_JS_P34
Machine: 3100
Run Time: 4/14/2005 - 13:27:40 -> 4/14/2005 - 14:17:43

Statistics	# Probes	Mean	StdDev
Control/Sample	6/41	0.86/1.14	0.29/0.36

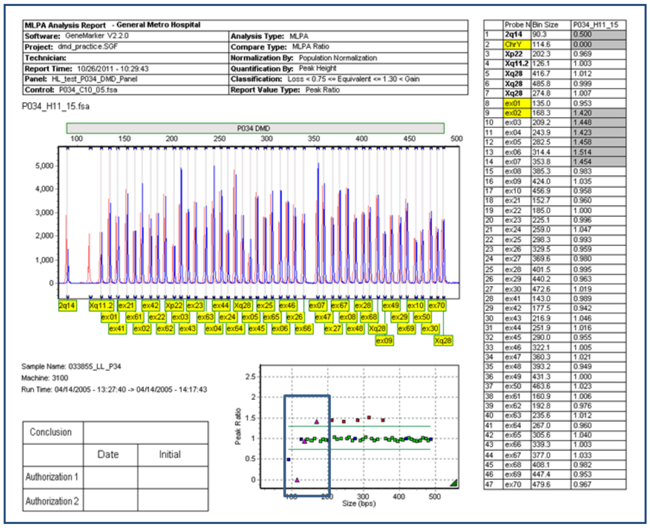
Conclusion		
	Date	Initial
Authorization 1		
Authorization 2		

	Probe Name	Bin Size	P034_C12_06
1	**2q14**	90.3	0.937
2	ChrY	114.6	0.947
3	**Xp22**	202.3	1.024
4	**Xq11.2**	126.1	1.027
5	**Xq28**	416.7	0.273
6	**Xq28**	485.8	0.916
7	**Xq28**	274.8	0.971
8	ex01	135.0	0.954
9	ex02	168.3	0.919
10	ex03	209.2	0.952
11	ex04	243.9	0.976
12	ex05	282.5	0.988
13	ex06	314.4	1.027
14	ex07	353.8	1.008
15	ex08	385.3	0.953
16	ex09	424.0	0.974
17	ex10	456.9	0.912
18	ex21	152.7	0.931
19	ex22	185.0	1.080
20	ex23	225.1	0.986
21	ex24	259.0	1.007
22	ex25	298.3	1.044
23	ex26	329.5	0.958
24	ex27	369.6	0.932
25	ex28	401.5	0.988
26	ex29	440.2	0.968
27	ex30	472.6	0.984
28	ex41	143.0	0.793
29	ex42	177.5	0.997
30	ex43	216.9	1.056
31	ex44	251.9	1.984
32	ex45	290.0	1.920
33	ex46	322.1	1.903
34	ex47	360.3	1.815
35	ex48	393.2	1.906
36	ex49	431.3	2.008
37	ex50	463.6	1.781
38	ex61	160.9	0.981
39	ex62	192.8	1.047
40	ex63	235.6	1.041
41	ex64	267.0	1.004
42	ex65	305.6	1.008
43	ex66	339.3	1.041
44	ex67	377.0	1.046
45	ex68	408.1	1.046
46	ex69	447.4	0.877
47	ex70	479.6	0.886

Electropherogram allele label options are set in the Main Analysis Window →View →Preferences → Display Settings →Chart Settings → Max Allele Label Layers. Select this option to display the allele labels in layers rather than the above view (which maximizes the peak display). If there are more than 46 samples a second page will be generated. Page option B4 allows up to 52 probes to be included on the first page of the report table.

Highlighting selected probes in the Final MLPA Report

In some chemistries, such as those that may contain pseudo genes, it is desirable to highlight certain alleles in the final report. Designate these alleles with [HL] in the comments column of the panel editor. The allele names that are designated [HL] will be highlighted in yellow in the final report table and represented as a fuchsia triangle in the ration plot.



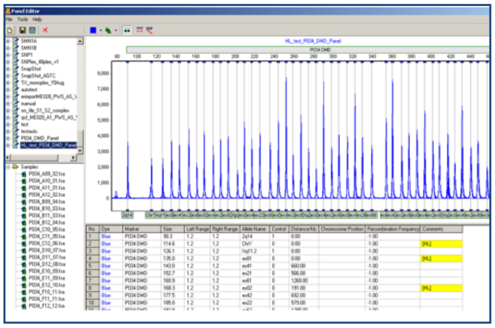
MLPA Analysis Report - General Metro Hospital	
Software: GeneMarker V2.2.0	**Analysis Type:** MLPA
Project: dmd_practice.SGF	**Compare Type:** MLPA Ratio
Technician:	**Normalization By:** Population Normalization
Report Time: 10/26/2011 - 10:29:43	**Quantification By:** Peak Height
Panel: HL_test_P034_DMD_Panel	**Classification:** Loss < 0.75 <= Equivalent <= 1.30 < Gain
Control: P034_C10_05.fsa	**Report Value Type:** Peak Ratio

P034_H11_15.fsa

Sample Name: 033855_LL_P34
Machine: 3100
Run Time: 04/14/2005 - 13:27:40 -> 04/14/2005 - 14:17:43

Conclusion		
	Date	Initial
Authorization 1		
Authorization 2		

	Probe N	Bin Size	P034_H11_15
1	**2q14**	90.3	0.500
2	ChrY	114.6	0.000
3	**Xp22**	202.3	0.969
4	**Xq11.2**	126.1	1.003
5	**Xq28**	416.7	1.012
6	**Xq28**	485.8	0.999
7	**Xq28**	274.8	1.007
8	ex01	135.0	0.953
9	ex02	168.3	1.420
10	ex03	209.2	1.448
11	ex04	243.9	1.423
12	ex05	282.5	1.458
13	ex06	314.4	1.514
14	ex07	353.8	1.454
15	ex08	385.3	0.983
16	ex09	424.0	1.035
17	ex10	456.9	0.958
18	ex21	152.7	0.960
19	ex22	185.0	1.000
20	ex23	225.1	0.996
21	ex24	259.0	1.047
22	ex25	298.3	0.993
23	ex26	329.5	0.959
24	ex27	369.6	0.980
25	ex28	401.5	0.995
26	ex29	440.2	0.963
27	ex30	472.6	1.019
28	ex41	143.0	0.989
29	ex42	177.5	0.942
30	ex43	216.9	1.046
31	ex44	251.9	1.016
32	ex45	290.0	0.955
33	ex46	322.1	1.005
34	ex47	360.3	1.021
35	ex48	393.2	0.949
36	ex49	431.3	1.000
37	ex50	463.6	1.023
38	ex61	160.9	1.006
39	ex62	192.8	0.976
40	ex63	235.6	1.012
41	ex64	267.0	0.960
42	ex65	305.6	1.040
43	ex66	339.3	1.003
44	ex67	377.0	1.033
45	ex68	408.1	0.982
46	ex69	447.4	0.953
47	ex70	479.6	0.967



MLPA Peak Height Normalization and Copy Number Determination

MLPA Data Normalization using Peak Heights

GeneMarker normalizes peak intensities based upon the statistically most probable median intensities (calculating median intensities helps determine the trend of the data). Median peak intensities are derived from the first five data points [GeneMarker v1.70 and later—GeneMarker v1.60 uses seven data points; GeneMarker v1.51 and earlier use nine data points], then sliding to data points 2-6, 3-7, etc., to ascertain the local median intensities. Outliers are rejected after applying a median filter. In order to correct for the peak intensity variation over size, an exponential function a*e-bz is used to fit to the square root (a statistical tool to reduce variation) of the peak intensities, where z is size, a and b are fitting constants. The normalized peak values are calculated using the exponential fit function and original peak height (intensity): Normalized Peak Value = Original peak intensity /Exponential fit peak intensity. The square roots of the intensity ratios are calculated and the ratios are plotted to model a linear regression. The median peak intensity is normalized to 3000.

Population Normalization reduces errors

Due to variations of PCR efficiencies from small to large DNA fragments or from sample to sample, two selectable normalization methods are provided. GeneMarker can normalize MLPA-derived data by an internal control probe method or a population method. The user is able to specify the normalization method in the Run Wizard (window three-Additional Settings) prior to analysis. Normalization using Control Probes removes the trend of dropping intensities as the DNA fragment size increases. However, the trend of peak intensities vary greatly from one sample to another with the internal control probes. Also, a small number of control probes or internal control probes that display atypical amplification kinetics often results in large errors in the intensity normalization. 'Population Normalization' addresses these problems, yielding better results. All of the probes (control and test) that are retained by the median filter are used to fit the exponential function, which results in higher accuracy and lower false positive rates.

MLPA Analysis using normalized peak heights and Control Sample(s)

When either MLPA Ratio or MLPA Regression Analyses are opened, the software has automatically selected a sample to use as the control (indicated by the # sign next to the sample). While the chosen sample may not actually be the experimental control, this selection is based upon which sample presents the data with the fewest abnormal calls; the sample with the least scatter of peak height ratios around the idealized ratio of 1.0 will be used as a control to calculate normalized height ratio of sample data to control data. The user may change the control to reflect their experimental sample or another sample which they feel portrays the data in a meaningful fashion.

GeneMarker software MLPA Ratio Analysis

In the MLPA analysis window, the normalized samples are compared to a control sample to determine duplications, deletions or no change. A t-test is used to determine which data points are outliers and which data points cluster together as the no-change data points.

MLPA Ratio plots the ratio of each sample compared to the control. The ratios of the sample data compared to the control sample [(control(s) defined by the user or software] are standardized such that the median point within the data set is considered to be 1. All other data points are relative to the median value. Outlying points are determined based upon the deviation of each allele peak relative to the average deviation of all peaks. Any individual peak whose residual deviation is greater than 3 times the average deviation will be considered outlying to the data. The outlying data points represent duplications and deletions. Duplications will be shown "above" the plotted ratio line and deletions will be "below" the line.

A color-coded triangle at the bottom right corner of each graph depicts the success of the standardization method for plotting of the peak ratios. A green triangle represents successful standardization; yellow represents standardization that needs to be verified for accuracy; red means standardization failed for that sample (or there are a significant number of probes displaying a copy number change).

Success or failure of the standardization is determined by calculating the relative deviation of the ratios. The probe ratio relative deviation calculation uses the following formula: [(75th percentile sample peak ratio/control peak ratio) – (25th percentile sample peak ratio/control peak ratio)] / (50th percentile sample peak ratio/control peak ratio).

If the probe ratio relative deviation is > 0.6, the triangle will be red. If the probe ratio relative deviation is > 0.4 < 0.6, the triangle will be yellow. If the probe ratio relative deviation is < 0.4, the triangle will be green.

GeneMarker software MLPA Regression Analysis

MLPA Regression analysis relies upon a T-Distribution to form the regression line between the square root of the sample and the square root of the control (using either peak heights or areas). GeneMarker utilizes an automatic iteration-based regression method. Using this method, the software forms a best-fit line, retaining 80% of peaks with smaller deviations and 20% are rejected. GeneMarker then iterates multiple times to reject and retain peaks, with a confidence of 99% (default setting – confidence interval denoted by green lines on graphs). This process is repeated until the regression line has reached the desired confidence. Finally, the removed data points are placed back into the plot, and will show up either on the regression line ("normal" copy number) or as outliers (possible copy number change).

Peak ratios (normalized peak value for test sample/normalized peak value for reference or control sample) are plotted on a patient-control graph. These peak ratios are plotted as representations of the square root of the sample and the square root of the control used to determine the best-fit line, outliers and confidence interval. The patient-control graph combines peak ratio information (to determine copy number changes) with the confidence interval from regression analysis.

Microsphere MLPA Analysis (Luminex)

An example of a DNA assay technique used to genotype patients with specific diseases is multiplex ligation-dependent probe amplification (MLPA). MLPA relies on sequence-specific probe hybridization to genomic DNA, followed by amplification of the hybridized probe, and analysis of the resulting PCR products. By labeling each set of hybridized probes with a unique microsphere, up to 100 distinct reactions can be multiplexed in a single reaction volume. Flow cytometry principles are used to allow microsphere tagged particles passing through a detection chamber to be measured discretely. Multiple MLPA assays can be developed in a single volume for detection of gene deletions and copy number changes.

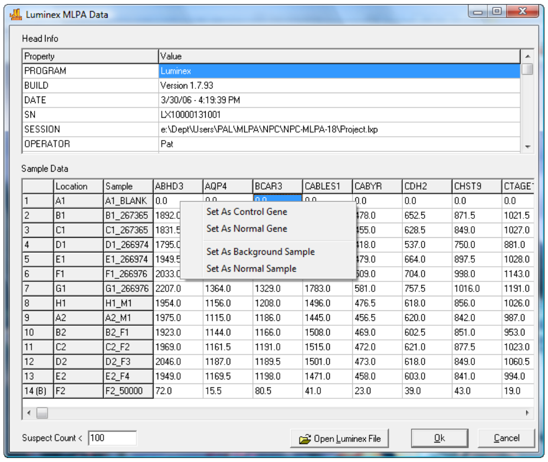
GeneMarker software has been developed to quickly and accurately analyze data from the Luminex® flow cytometry instruments (Luminex 100 IS and Luminex 200) for microsphere detection. The software is therefore compatible with Luminex instrument data format (.csv). With GeneMarker, MLPA microsphere data analysis is a quick and accurate analysis with customizable features including reports and printing.

Overview

The steps required to analyze Luminex instrument data after MLPA are different from other analyses done by GeneMarker. Due to the nature of microsphere labeling, peak intensities are artificial and represent the number of beads detected by the flow cytometer. The software normalizes to an arbitrary 3000 using a combination method. Because the Luminex data is different than standard capillary electrophoresis data, a size standard and panel do not need to be applied.

Procedure

1. Open GeneMarker Software
2. Close *Start Your Project* box
3. Go to **Tools → Luminex MLPA Analysis**
4. The *Open Luminex Data* box appears
5. Select a **CSV** file to analyze
6. Click **Open**
7. The *Luminex MLPA Data* window appears
   a. Alleles = Columns
   b. Samples = Rows
   c. Right-click on a row to select the *Background* sample. The software automatically chooses the overall least intense sample and labels it with a **(B)**.
   d. Right-click on a column to select the *Control Gene*. If you do not select control gene then the software will automatically select the best control given your data set.
   e. *Suspect Count* in the bottom left corner is set to default **100**. Suspect Count represents the threshold for bead number – any sample with less than the Suspect Count threshold will be flagged for user inspection.



Head Info

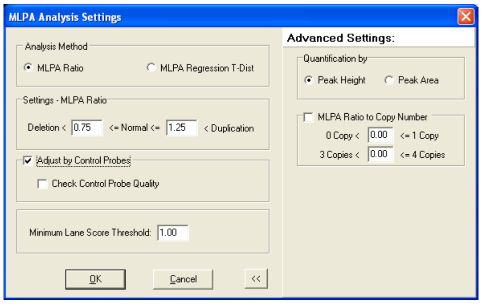
Property	Value
PROGRAM	Luminex
BUILD	Version 1.7.93
DATE	3/30/06 - 4:19:39 PM
SN	LX10000131001
SESSION	e:\Dept\Users\PAL\MLPA\NPC\NPC-MLPA-18\Project.lxp
OPERATOR	Pat

Sample Data

	Location	Sample	ABHD3	AQP4	BCAR3	CABLES1	CABYR	CDH2	CHST9	CTAGE
1	A1	A1_BLANK	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2	B1	B1_267365	1892.0				78.0	652.5	871.5	1021.5
3	C1	C1_267365	1831.5				55.0	628.5	849.0	1027.0
4	D1	D1_266974	1795.0				18.0	537.0	750.0	881.0
5	E1	E1_266974	1949.5				79.0	664.0	897.5	1028.0
6	F1	F1_266976	2033.0				09.0	704.0	998.0	1143.0
7	G1	G1_266976	2207.0	1364.0	1329.0	1783.0	581.0	757.5	1016.0	1191.0
8	H1	H1_M1	1954.0	1156.0	1208.0	1496.0	476.5	618.0	856.0	1026.0
9	A2	A2_M1	1975.0	1115.0	1186.0	1445.0	456.5	620.0	842.0	987.0
10	B2	B2_F1	1923.0	1144.0	1166.0	1508.0	469.0	602.5	851.0	953.0
11	C2	C2_F2	1969.0	1161.5	1191.0	1515.0	472.0	621.0	877.5	1023.0
12	D2	D2_F3	2046.0	1187.0	1189.5	1501.0	473.0	618.0	849.0	1060.5
13	E2	E2_F4	1949.0	1169.5	1198.0	1471.0	458.0	603.0	841.0	994.0
14 (B)	F2	F2_50000	72.0	15.5	80.5	41.0	23.0	39.0	43.0	19.0

Suspect Count < 100

8. Click **OK** in the *Luminex MLPA Data* window
9. The *MLPA Analysis Settings* window opens
10. Recommended Settings:
    a. Analysis Method: **MLPA Ratio**
    b. Settings – MLPA Ratio:
    c. Deletion < **0.75** <= Normal <= **1.30** < Duplication
    d. Minimum Lane Score Threshold: **10.00**
    e. Quantification by: **Peak Height**
11. Click **OK**
12. The *MLPA Analysis* window appears



Analysis Method
● MLPA Ratio ○ MLPA Regression T-Dist

Settings - MLPA Ratio
Deletion < 0.75 <= Normal <= 1.25 < Duplication

☑ Adjust by Control Probes
☐ Check Control Probe Quality

Minimum Lane Score Threshold: 1.00

Advanced Settings:

Quantification by
● Peak Height ○ Peak Area

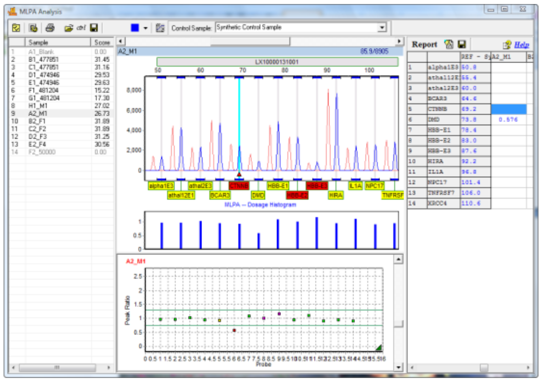
☐ MLPA Ratio to Copy Number
0 Copy < 0.00 <= 1 Copy
3 Copies < 0.00 <= 4 Copies

What to Expect

Luminex Data Normalization

Due to differences in laboratory requirements, GeneMarker has the option to normalize MLPA-derived data in two different ways, by an internal control probe method or by a population method. The user is able to specify the normalization method by selecting a control sample in the *Luminex MLPA Data* window or in the *MLPA Analysis* window's *Control Sample* drop-down menu (select *Synthetic Control Sample* to use population normalization).

Luminex Data Analysis



After Control Genes and the Background Sample are selected in the *Luminex MLPA Data* window, the MLPA Analysis window appears. See the *MLPA Analysis* section above for more information about icons and functions.

A feature unique to the Luminex MLPA Analysis window is that samples that contain probes that do not meet the minimum bead threshold, or *Suspect Count*, are identified. Suspect probes are labeled in red in the *Overlay Trace* and their data points appear fuchsia in the *Ratio Plot*. MLPA data analysis proceeds as outlined in the *MLPA Analysis* section above.

Methylation-Specific MLPA Analysis (MS-MLPA)

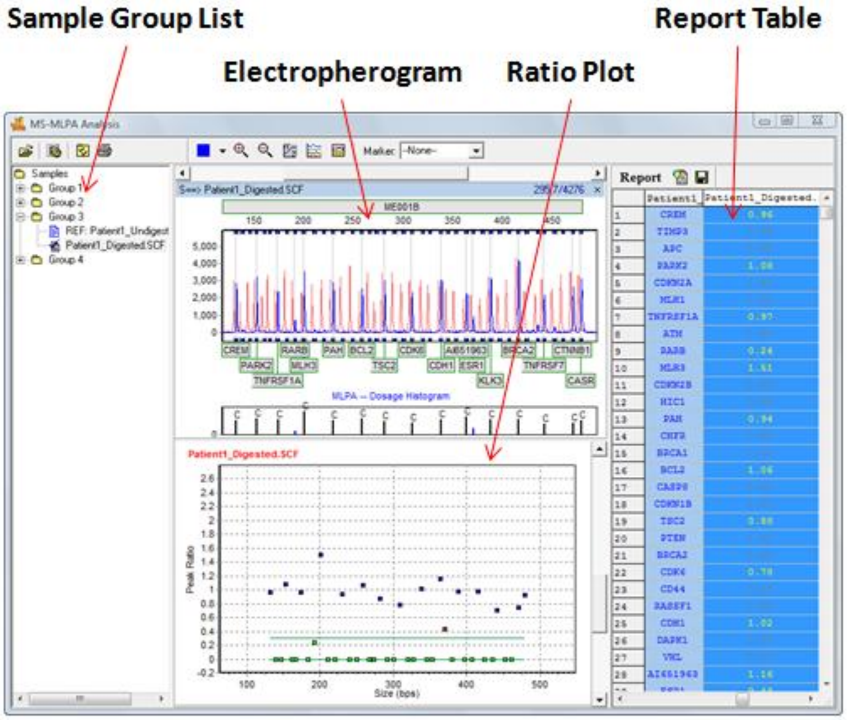
Detection of methylated sites in genomic DNA is important for two reasons. First, methylated DNA locations cannot be transcribed. This is important when the methylation occurs in the promoter region of genes. If the promoter region of a gene is methylated, the gene will not be transcribed and its associated protein will not be produced. Methylation of the promoter region for tumor suppressor genes is of great interest for cancer diagnostics. When the tumor suppressor gene is switched off because of methylation in the promoter region, the cell's growth will be unchecked and cancer may form. Likewise, if methylation occurs in mismatch repair gene promoter regions, genetic mutations within a cell will proliferate, thereby increasing the risk of tumorigenesis.

Second, DNA methylation is important in discovering cases of genomic imprinting. At conception, two copies of each chromosome are inherited from an individual's parents, one copy from the father and one copy from the mother. Normally, it cannot be determined from which parent each chromosome originated; however, in some cases, differential expression of a gene occurs depending upon which parental chromosome the gene resides. Additional research linked differential expression of genes with methylation patterns and/or histone modifications around the gene of interest. Methylation is useful in determining parental chromosome origin and is referred to as genomic imprinting.

Overview

GeneMarker DNA analysis software has been successfully paired with the Multiplex Ligation-dependent Probe Amplification (MLPA) technique for detecting genetic deletions and duplications in various diseases including cancer. Recently, the MLPA technique has been improved to detect methylation sites within promoter regions and for genomic imprinting applications. Promoter Methylation kits from MRC-Holland include ME001B Tumor Suppressor, ME002 Tumor Suppressor, and ME011 for Mismatch Repair genes. Genomic Imprinting kits from MRC-Holland include ME028 PWS-AS and ME030 BWS-RSS. GeneMarker's new Methylation Specific – MLPA (MS-MLPA) module quickly and accurately detects methylation sites for researchers studying promoter methylation and genomic imprinting diseases. GeneMarker's ease of use and professional reporting options are an excellent choice for MS-MLPA applications.

MS-MLPA



	Patient1_	Patient1_Digested.
1	CREM	0.96
2	TIMP3	
3	APC	
4	PARK2	1.08
5	CDKN2A	
6	MLH1	
7	TNFRSF1A	0.97
8	ATM	
9	RARB	0.24
10	MLH3	1.51
11	CDKN2B	
12	HIC1	
13	PAH	0.94
14	CHFR	
15	BRCA1	
16	BCL2	1.06
17	CASP8	
18	CDKN1B	
19	TSC2	0.88
20	PTEN	
21	BRCA2	
22	CDK6	0.78
23	CD44	
24	RASSF1	
25	CDH1	1.02
26	DAPK1	
27	VHL	
28	AI651963	1.16

Sample Group List

Samples in the *Sample Group List* appear within Group folders according to the information provided in the *File Name Group Text* file uploaded in the *MS-MLPA Analysis Settings* box. A sample is marked as the Reference because it contains a *Control Identifier* as set in the *File Name Group* tool. **See Chapter 8 Additional Tools**. Reference/Control samples will appear in the first column of the *File Name Group Text* file.

Expand folders in the *Sample Group List* to view the Reference sample and Experimental sample in the group. Double-click the Reference sample to view just the Reference sample electropherogram. Double-click the Experimental sample to view the Experimental sample electropherogram overlaid on the Reference sample electropherogram and associated *Dosage Histogram*.

Electropherogram

The *Electropherogram* displays a normalized electropherogram trace of the selected sample with the control sample reference trace displayed in light red behind it. Change the degree of shift between the selected sample and the reference trace in the *MS-MLPA Display Settings* box. The allele or exon names are displayed below their corresponding bins.

The *Dosage Histogram* displays, in bar chart form, the ratio of normalized peak intensities between the reference and the sample trace. Sample probes are represented by blue bars and control probes are black bars.

Allele editing options in the *Trace Overlay* frame are similar to those in the *Main Analysis* window *Electropherogram*. **See Chapter 3 Main Analysis Overview**.

Ratio Plot

The *Ratio Plot* displays, in graphical form, the ratio of normalized peak intensities between the reference and the sample trace. Each point represents a sample peak or probe. Enter the number of *Ratio Plots* to display simultaneously in the *MS-MLPA Display Settings* box.

Green = Peak is within the specified ratio limits
Red = Peak is outside the specified ratio limits
Blue = Peak is a control probe
Yellow = Current selected probe

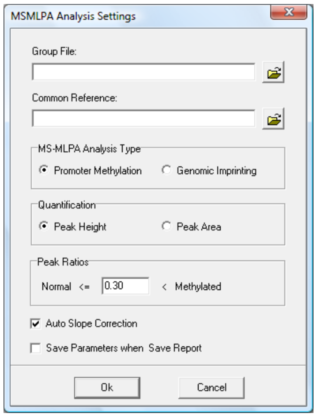
Double-click a point in the *Ratio Plot* to view the peak in the *Electropherogram* and the ratio value in the *Report Table*.

Right-click in the *Ratio Plot* and select *Adjust by Control Probes* to fit the data by only the control probes (blue points) in the sample. Right-click and select *Reset* to restore population fit calculation (all control and sample probes in the sample are used to fit the data).

Report Table

The *Report Table* lists in columns the probe or exon name (according to the panel selected) and the ratio for each sample probe as compared to the reference. Double-click a cell in the *Report Table* to display the corresponding peak in the *Electropherogram* and the plot point in the *Ratio Plot*. See the *Reports and Printing* section below for additional options in the *Report Table*.

Procedure


MSMLPA Analysis Settings

Group File:

Common Reference:

MS-MLPA Analysis Type
● Promoter Methylation ○ Genomic Imprinting

Quantification
● Peak Height ○ Peak Area

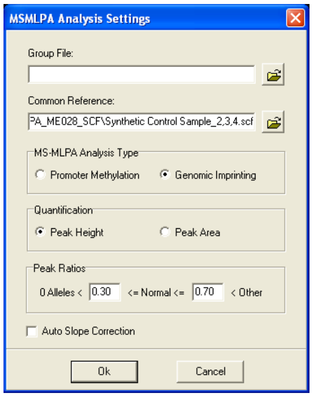
Peak Ratios
Normal <= 0.30 < Methylated

☑ Auto Slope Correction
☐ Save Parameters when Save Report

1. Launch *GeneMarker* software
2. Import raw capillary electrophoresis data
3. Apply size standard and peak filtering options with *Run Wizard*
4. Select a panel in *Panel Editor* and align Markers and Bins accordingly
5. Select **Applications → MS-MLPA**
6. The *MS-MLPA Analysis Settings* box will appear
7. Import grouping information created in the *Filename Group Tool* into the *Group File* field, or import Common Reference [SG1 (5-color data), SCF (4-color data), FSA file format] into the *Common Reference* field, or enter both a *Group File* and *Common Reference*. A synthetic reference or control may be constructed and saved using this function in the MLPA analysis window (see above). A Common or Synthetic Reference must be imported into the project to be available for use (click **Project** in the menu bar and select **Add**

Samples to Project).

8. Select an *MS-MLPA Analysis Type*


MSMLPA Analysis Settings

Group File:

Common Reference: PA_ME028_SCF\Synthetic Control Sample_2,3,4.scf

MS-MLPA Analysis Type: ○ Promoter Methylation ● Genomic Imprinting

Quantification: ● Peak Height ○ Peak Area

Peak Ratios: 0 Alleles < 0.30 <= Normal <= 0.70 < Other

☐ Auto Slope Correction

Promoter Methylation

9. Select *Promoter Methylation Analysis Type*
10. Choose to analyze by *Peak Height* *OR* *Peak Area*
11. Enter the *Peak Ratio* value below which a peak will not be considered methylated
12. Click **OK**
13. Analyze data for non-control peaks above the *Methylated Peak Ratio* set in the *Analysis Settings* box
14. Click the **Print** icon to create an *MS-MLPA Clinical Report*

Genomic Imprinting

9. Select *Genomic Imprinting Analysis Type*
10. Choose to analyze by *Peak Height* *OR* *Peak Area*
11. Enter the *Peak Ratio* values between which a peak will be considered normal
12. Click **OK**
13. Analyze data for non-control peaks above the *Other Peak Ratio* set in the *Analysis Settings* box
14. Click the **Print** icon to create an *MS-MLPA Clinical Report*

What to Expect

The MRC-Holland MS-MLPA kits take advantage of methylation sensitive endonucleases (HhaI and HpaII) which will cleave unmethylated DNA fragments. Methylated fragments are not digested by the endonucleases and will therefore produce a peak signal in the electropherogram. In addition to novel methylation detection probes, MRC-Holland includes multiple control probe genes in the MS-MLPA kit for monitoring the efficiency of the endonuclease activity.

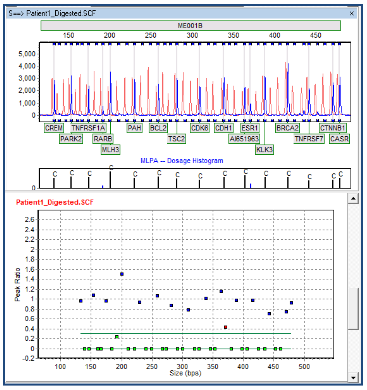
Promoter Methylation

In trace electropherogram, the undigested patient DNA sample appears as the red background trace in the electropherogram and is used as the reference. The same patient's digested DNA sample appears as the blue trace in the electropherogram. Based on the *Quantification* method chosen in the *MS-MLPA Analysis Settings* box (*Peak Height* or *Peak Area*); the digested sample trace is compared to the undigested reference trace. The ratio value of the comparison is plotted by basepair size in the *Ratio Plot* below the electropherogram.

In the *Ratio Plot*, the blue plot points represent the control probe peak ratios. As expected, the control probes of the sample trace are in a 1:1 ratio with the control probe peaks of the reference trace. A green threshold line appears at Ratio = 0.3 and represents the Methylated Peak Ratio value set in the MS-MLPA Analysis Settings box. Green plot points below this threshold do not contain a significant peak in the sample trace compared to the reference trace because the fragment was digested by the endonuclease. Since the fragment was digested by the endonuclease, it is assumed the gene is unmethylated. Red plot points that appear above the green threshold line represent an amplified undigested fragment. Undigested fragments, which are not control probes, are considered sites of methylation. In

Figure 1, one copy of gene ESR1 is methylated because its representative red plot point appears above the Methylated Peak Ratio threshold line at around Ratio = 0.5.

Promoter Methylation


S==> Patient1_Digested.SCF
ME001B
150
200
250
300
350
400
450
5,000
4,000
3,000
2,000
1,000
0
CREM
TNFRSF1A
PARK2
RARB
MLH3
PAH
BCL2
TSC2
CDK6
CDH1
ESR1
AI651963
KLK3
BRCA2
TNFRSF7
CTNNB1
CASR
MLPA -- Dosage Histogram
Patient1_Digested.SCF
Peak Ratio
2.6
2.4
2.2
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
-0.2
100
150
200
250
300
350
400
450
500
Size (bps)

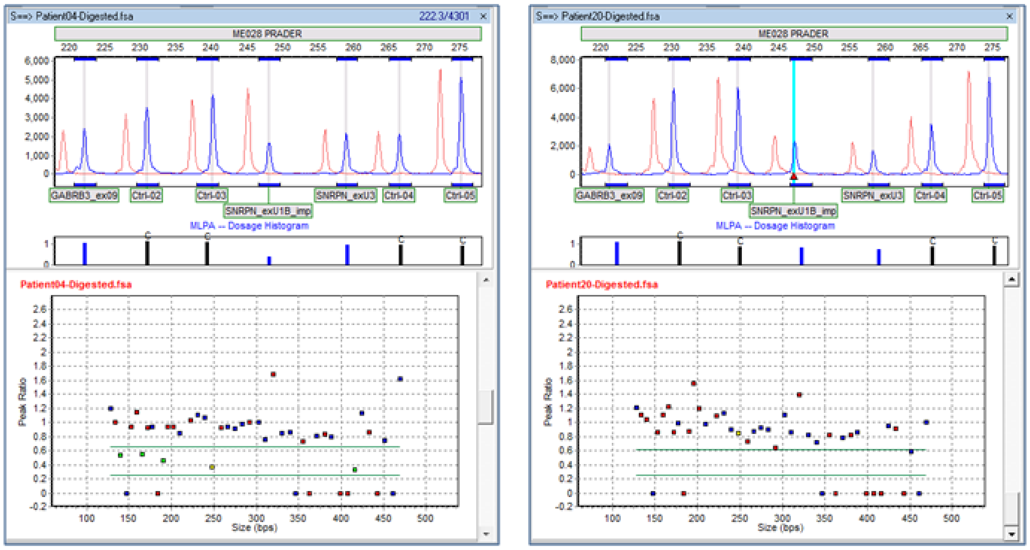
Genomic Imprinting

Prior to detecting genomic imprinting with MS-MLPA, a researcher must determine if the genes of interested have been deleted with the traditional MLPA technique. Once the copy number has been determined, digestion with methylation sensitive endonucleases can be performed to detect imprinting.

In the example below, a normal patient's sample appears on the left and a sample from a patient suffering from Prader-Willi Syndrome appears on the right. In the normal patient's sample, five green plot points appear between the green methylation threshold lines. These plot points represent five genes of interest located in the PWS/AS region on chromosome 15. As discussed previously, unmethylated fragments are completely digested by the methylation sensitive endonuclease and methylated fragments remain undigested. The five plot points that appear around Ratio = 0.5 represent genes that contain one methylated allele and one unmethylated allele. A normal patient, without a deletion at the PWS/AS site, will express both the methylated and unmethylated genes from the mother and father, respectively.

In the sample from the patient suffering from PWS, none of the five genes of interest plot points appear between the methylation threshold lines. Upon further inspection, it is determined that the five peaks are in a 1:1 ratio with the undigested fragments. Because it was determined previously with traditional MLPA analysis that this patient's PWS/AS region contained a deletion on one chromosome, only the other parent's chromosome genes are represented by the fragment peak. In the PWS case, since the remaining gene fragments were not digested, it is deduced that the remaining chromosome is methylated and therefore comes from the mother. The father's PWS/AS genes have been deleted. For a patient suffering from Angelman Syndrome, the mother's PWS/AS genes would be deleted leaving the paternal chromosome genes. The paternal chromosome is unmethylated in the PWS/AS region; therefore the fragments would be completely digested and no peak would appear in the electropherogram.

Genomic Imprinting


S==> Patient04-Digested.fsa
222.3/4301
S==> Patient20-Digested.fsa
ME028 PRADER
GABRB3_ex09
Ctrl-02
Ctrl-03
SNRPN_exU1B_imp
SNRPN_exU3
Ctrl-04
Ctrl-05
MLPA -- Dosage Histogram
Patient04-Digested.fsa
Patient20-Digested.fsa
Peak Ratio
Size (bps)

Reports and Printing

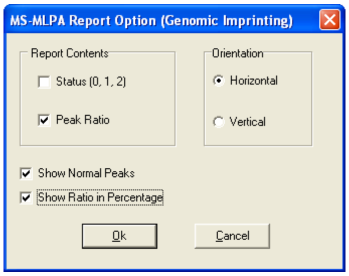
GeneMarker automates the analysis process and creates professional clinical reports for accurate insertion/deletion detection.

Report Table

Displays peak ratio information for individual peaks of a sample compared to its designated reference. A sample column will be highlighted blue when the sample is selected in the *Sample Group List*. The *Electropherogram* trace will correspond to which ever peak cell is selected in the *Report Table*.

Report Settings

Launches the *MS-MLPA Report Options* dialog box.


MS-MLPA Report Option (Genomic Imprinting)

Report Contents
☐ Status (0, 1, 2)
☑ Peak Ratio

Orientation
● Horizontal
○ Vertical

☑ Show Normal Peaks
☑ Show Ratio in Percentage

Report Contents: Selecting *Status (-,+)* will place a "–" or "+" sign in the cell for each detected allele to represent the presence or absence of alleles, respectively. Selecting Peak Ratio will display the ratio value of the sample peak height or area compared to the reference peak.

Orientation: *Horizontal* orientation displays the *Report Table* with samples listed in a column on the left and allele peak ratio values in rows. *Vertical* orientation displays samples in a row along the top of the *Report Table* and allele peak ratio values in columns.

Show Normal Peaks: Displays the peak height ratios of peaks within the methylation thresholds set in the *MS-MLPA Analysis Settings* box.

Show Ratio in Percentage: Displays peak height ratios as percentages.

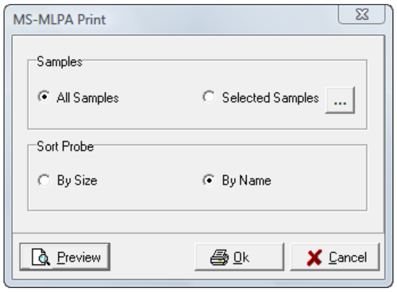
Save Parameters when Save Report: Automatically generates an *.ini file of the MS-MLPA Analysis Parameters when the MS-MLPA report is saved. Naming convention for the file is Report file name_MS_MLPA_Settings.ini

Save Report

Choose to save the *Report Table* as an Excel file (.xls) or a tab-delimited Text file (.txt).

MS-MLPA Print ReportSettings

Click the **Print Report** icon to launch the *MS-MLPA Print* dialog box and preview, print or save the *MS-MLPA Print Report*.



Samples
● All Samples ○ Selected Samples ...

Sort Probe
○ By Size ● By Name

Samples

Select *All Samples* or just the samples you wish to report. To *Select Samples*, click the "…" button next to *Selected Samples* and place a check mark in the box next to the samples you wish to appear in the report.

Sort Probe

Sort the probes in descending order *By Size* or *By Name* in the *Methylation Report Table*.

MS-MLPA Print Report

Below is a description of the *MS-MLPA Print Report* features. For an explanation of functions within the *Print Preview* window, see **Chapter 6 Reports and Printing**.

Report Header

Contains information about the analysis, project, sample and parameters.

Electropherogram

Similar to the *MS-MLPA Analysis* window, displays the reference trace in light red behind the sample trace (dye color). The zoom setting in the *MS-MLPA Analysis* window is applied in the *Print Report*.

Ratio Plot

Similar to the *MS-MLPA Analysis* window, the plot points indicate the Peak Height/Area Ratio of sample to reference.

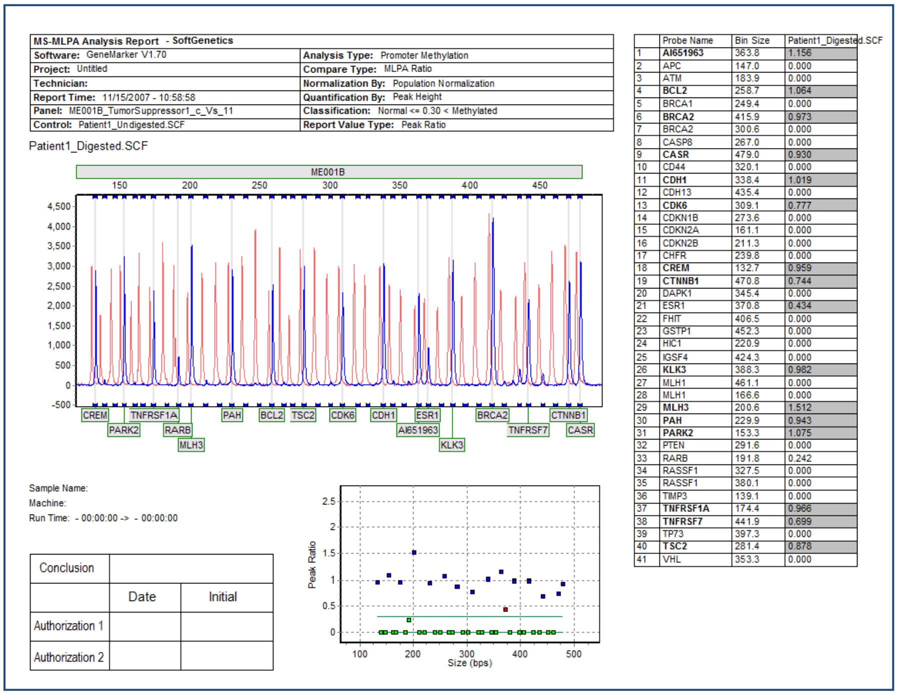
Signature Box

Date and initial space for report reviewers.

Methylation Report Table

A list of all probe's peak ratio information. Peak ratio cells which meet the methylation threshold criteria are highlighted grey. Control probes are in bold font.

MS-MLPA Print Report



MS-MLPA Analysis Report - SoftGenetics	
Software: GeneMarker V1.70	**Analysis Type:** Promoter Methylation
Project: Untitled	**Compare Type:** MLPA Ratio
Technician:	**Normalization By:** Population Normalization
Report Time: 11/15/2007 - 10:58:58	**Quantification By:** Peak Height
Panel: ME001B_TumorSuppressor1_c_Vs_11	**Classification:** Normal <= 0.30 < Methylated
Control: Patient1_Undigested.SCF	**Report Value Type:** Peak Ratio

Patient1_Digested.SCF

Sample Name:
Machine:
Run Time: - 00:00:00 -> - 00:00:00

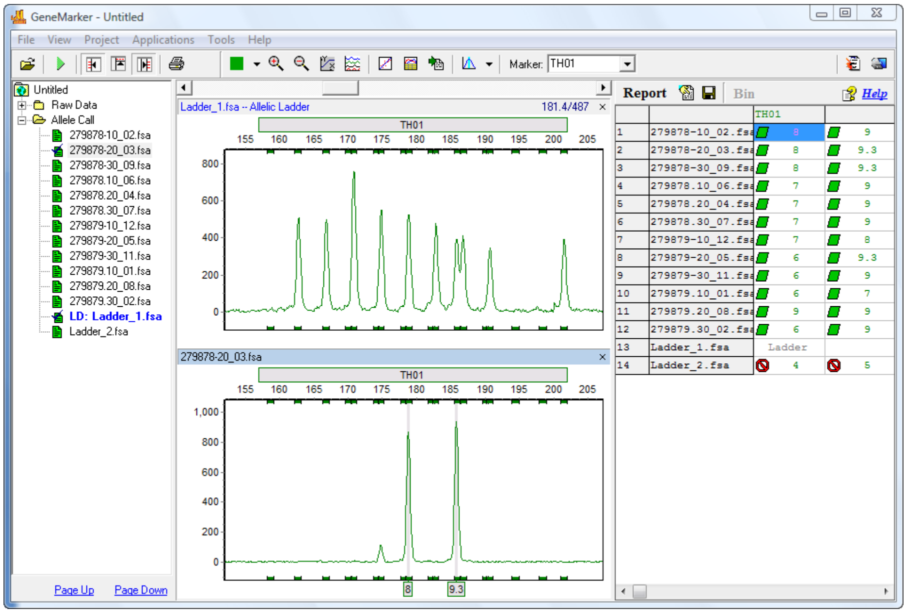
Conclusion		
	Date	Initial
Authorization 1		
Authorization 2		

	Probe Name	Bin Size	Patient1_Digested.SCF
1	**AI651963**	363.8	1.156
2	APC	147.0	0.000
3	ATM	183.9	0.000
4	**BCL2**	258.7	1.064
5	BRCA1	249.4	0.000
6	**BRCA2**	415.9	0.973
7	BRCA2	300.6	0.000
8	CASP8	267.0	0.000
9	**CASR**	479.0	0.930
10	CD44	320.1	0.000
11	**CDH1**	338.4	1.019
12	CDH13	435.4	0.000
13	**CDK6**	309.1	0.777
14	CDKN1B	273.6	0.000
15	CDKN2A	161.1	0.000
16	CDKN2B	211.3	0.000
17	CHFR	239.8	0.000
18	**CREM**	132.7	0.959
19	**CTNNB1**	470.8	0.744
20	DAPK1	345.4	0.000
21	ESR1	370.8	0.434
22	FHIT	406.5	0.000
23	GSTP1	452.3	0.000
24	HIC1	220.9	0.000
25	IGSF4	424.3	0.000
26	**KLK3**	388.3	0.982
27	MLH1	461.1	0.000
28	MLH1	166.6	0.000
29	**MLH3**	200.6	1.512
30	**PAH**	229.9	0.943
31	**PARK2**	153.3	1.075
32	PTEN	291.6	0.000
33	RARB	191.8	0.242
34	RASSF1	327.5	0.000
35	RASSF1	380.1	0.000
36	TIMP3	139.1	0.000
37	**TNFRSF1A**	174.4	0.966
38	**TNFRSF7**	441.9	0.699
39	TP73	397.3	0.000
40	**TSC2**	281.4	0.878
41	VHL	353.3	0.000

Human Identity (HID)

The *Human Identity (HID) Analysis Type* in GeneMarker was designed for use with forensic short tandem repeat (STR) data. The forensic community recognizes approximately 18 "core" loci by which humans can be tested and typed. The core loci were selected for human typing because they follow the Mendelian laws of segregation and independent assortment, they typically do not code for any particular protein, and they are highly variable in the population. Tetra and penta (4 and 5) nucleotide repeat units are also characteristic of the forensic core loci. Several commercially available human typing kits are available from ABI and Promega and are included as pre-defined Panels in GeneMarker. For advanced human identification analysis please try GeneMarker® HID software. Post bone marrow transplant (chimerism) analysis and monitoring studies also use Human Identification PCR kits. ChimerMarker™ software provides Short Tandem Repeat analysis, automated chimerism analysis and monitoring. Fully functional validation versions of the programs may be downloaded from http://www.softgenetics.com/downloads.html

Human Identity Analysis


GeneMarker - Untitled
File
View
Project
Applications
Tools
Help
Marker: TH01
Untitled
Raw Data
Allele Call
279878-10_02.fsa
279878-20_03.fsa
279878-30_09.fsa
279878.10_06.fsa
279878.20_04.fsa
279878.30_07.fsa
279879-10_12.fsa
279879-20_05.fsa
279879-30_11.fsa
279879.10_01.fsa
279879.20_08.fsa
279879.30_02.fsa
LD: Ladder_1.fsa
Ladder_2.fsa
Page Up
Page Down
Ladder_1.fsa -- Allelic Ladder
181.4/487
TH01
155
160
165
170
175
180
185
190
195
200
205
800
600
400
200
0
279878-20_03.fsa
TH01
155
160
165
170
175
180
185
190
195
200
205
1,000
800
600
400
200
0
8
9.3
Report
Bin
Help

		TH01	
1	279878-10_02.fsa	🟩 8	🟩 9
2	279878-20_03.fsa	🟩 8	🟩 9.3
3	279878-30_09.fsa	🟩 8	🟩 9.3
4	279878.10_06.fsa	🟩 7	🟩 9
5	279878.20_04.fsa	🟩 7	🟩 9
6	279878.30_07.fsa	🟩 7	🟩 9
7	279879-10_12.fsa	🟩 7	🟩 8
8	279879-20_05.fsa	🟩 6	🟩 9.3
9	279879-30_11.fsa	🟩 6	🟩 9
10	279879.10_01.fsa	🟩 6	🟩 7
11	279879.20_08.fsa	🟩 9	🟩 9
12	279879.30_02.fsa	🟩 6	🟩 9
13	Ladder_1.fsa	Ladder	
14	Ladder_2.fsa	🚫 4	🚫 5

Procedure

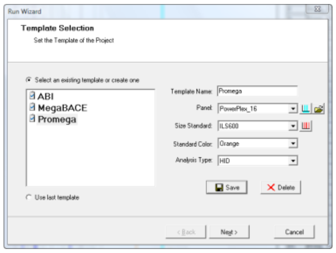
HID analysis in GeneMarker is similar to Fragment (Animal) analysis with the exception that the Stutter Filter and Peak Score thresholds have been lowered to allow more peaks to be called. In the Run Wizard *Template Selection* box, be sure to choose the correct Size Standard, and set the *Analysis Type* as **HID**. Additionally, forensic datasets generally contain 2 or more Allelic Ladder samples by which the forensic Panel can be adjusted.

HID Run Wizard Settings

For explanation of specific functions in Run Wizard, **see Chapter 2 General Procedure**. The following are the default settings for *HID Analysis Type*.

Run Wizard Template Selection

Panel: Select the Panel that matches the Allelic Ladder samples
Size Standard: User-defined
Analysis Type: HID

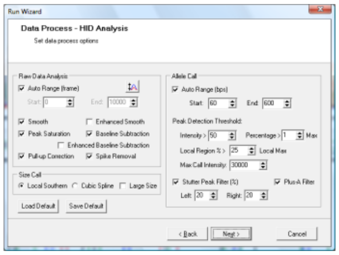

Run Wizard
Template Selection
Set the Template of the Project
ABI
MegaBACE
Promega
Save
Delete
< Back
Next >
Cancel

● Select an existing template or create one
○ Use last template

Template Name: Promega
Panel: PowerPlex_16
Size Standard: ILS600
Standard Color: Orange
Analysis Type: HID

Run Wizard Data Process

Peak Detection Intensity Threshold: 50
Global Percentage: 1% Max


Run Wizard
Data Process - HID Analysis
Set data process options
Raw Data Analysis
Allele Call
Size Call
Load Default
Save Default
< Back
Next >
Cancel

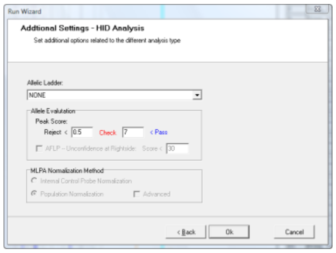
☑ Auto Range (frame)
Start: 0 End: 10000
☑ Smooth ☐ Enhanced Smooth
☑ Peak Saturation ☑ Baseline Subtraction
☐ Enhanced Baseline Subtraction
☑ Pull-up Correction ☑ Spike Removal
● Local Southern ○ Cubic Spline ☐ Large Size
☑ Auto Range (bps)
Start: 60 End: 600
Peak Detection Threshold:
Intensity > 50 Percentage > 1 Max
Local Region % > 25 Local Max
Max Call Intensity: 30000
☑ Stutter Peak Filter (%) ☑ Plus-A Filter
Left: 20 Right: 20

Local Percentage: 25%
Max Call Intensity: 30,000
Stutter Peak Filter: *Left:* 20% *Right:* 20%
Plus A Filter: Selected

Run Wizard Additional Settings

Allelic Ladder: Select an Allelic Ladder sample from the dataset drop-down list
Allele Evaluation Peak Score: Reject < 0.5 Check 7 < Pass

NOTE: If the Reject value is set too high, a false negative call could result. If the Reject value is set too low, then a false positive call may occur.


Run Wizard
Addtional Settings - HID Analysis
Set additional options related to the different analysis type
< Back
Ok
Cancel

Allelic Ladder: NONE

Allele Evalutation
Peak Score:
Reject < 0.5 Check 7 < Pass
☐ AFLP -- Unconfidence at Rightside: Score < 30

MLPA Normalization Method
○ Internal Control Probe Normalization
● Population Normalization ☐ Advanced

HID Panel Adjustment

After the data has been sized and filters have been applied, go to *Tools* → *Panel Editor* to adjust the selected Panel for the dataset. For explanation of specific functions in *Panel Editor*, **see Chapter 5 Panel Editor**.

1. In *Panel Editor*, select the appropriate forensic Panel from the *Panel List*
2. The select Panel will appear in the *Overlay Trace* frame
3. Click the **Trace Mode** icon until the *Trace Overlay* view appears in the *Trace Overlay* frame
4. Right-click in the *Samples List* and select *Deselect All* so that no samples are selected
5. Double-click only the Allelic Ladder samples in the *Samples List*
6. Click the **Major Adjustment of Panel** icon
7. Click the **Minor Adjustment of Panel** icon
8. The Markers and Bins will shift to align with the average peak positions of the Allelic Ladder samples
9. Verify Marker and Bin shift was accurate by clicking the **Show Dye** icon
    a. Hold down **SHIFT** key and left-click and drag Markers/Bins to align correctly with the Allelic Ladders
10. After editing, select *File* → *Save Changes with Signal Info* OR click **Save Changes with Signal Info** icon
11. Exit the *Panel Editor*
12. Click the **Run Process** icon in the *Main Analysis* window
13. Select the newly adjusted Panel from the Run Wizard *Template Selection Panel* drop-down menu
14. Proceed through Run Wizard and click **OK** in the *Data Process* box
15. The Panel has been applied

What to Expect

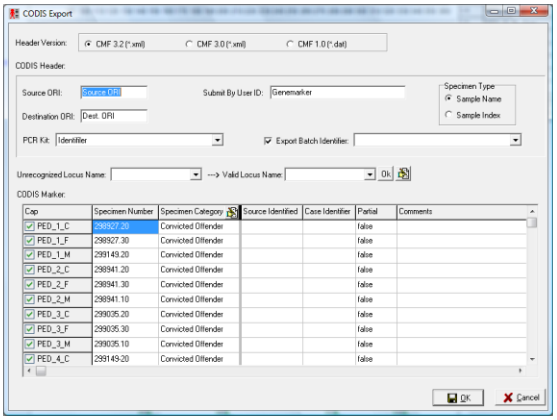
When HID Analysis Type is selected in Run Wizard, the *Main Analysis* window automatically sets the selected Allelic Ladder sample as the top *Electropherogram* and the *Report Table* displays *Marker Table (Fragment) Report Style*. Use the *Marker* drop-down box to scroll through loci or click the **All Color Browser** icon to view all dye colors of a sample simultaneously. Edit alleles as required. **See Chapter 3 Main Analysis Overview**. Click the **Print** icon to print the results. **See Chapter 6 Reports and Printing**.

CODIS Report

The *Export CODIS* tool was created for forensic labs that need to create reports for upload into the Federal Bureau of Investigation's Combined DNA Index System (CODIS).

To access the CODIS report, select *Applications* → *Export CODIS.*

This tool allows the user to select profile descriptions from a drop-down list for each individual sample and save the report as a CMF 3.2, CMF 3.0 (.xml) file and CMF 1.0 (.dat) file.



Header Version: ● CMF 3.2 (*.xml) ○ CMF 3.0 (*.xml) ○ CMF 1.0 (*.dat)

Source ORI: Source ORI
Submit By User ID: Genemarker
Destination ORI: Dest. ORI
Specimen Type: ● Sample Name ○ Sample Index
PCR Kit: Identifiler
☑ Export Batch Identifier:

CODIS Marker:

Cap	Specimen Number	Specimen Category	Source Identified	Case Identifier	Partial	Comments
☑ PED_1_C	298927.20	Convicted Offender			false	
☑ PED_1_F	298927.30	Convicted Offender			false	
☑ PED_1_M	299149.20	Convicted Offender			false	
☑ PED_2_C	298941.20	Convicted Offender			false	
☑ PED_2_F	298941.30	Convicted Offender			false	
☑ PED_2_M	298941.10	Convicted Offender			false	
☑ PED_3_C	299035.20	Convicted Offender			false	
☑ PED_3_F	299035.30	Convicted Offender			false	
☑ PED_3_M	299035.10	Convicted Offender			false	
☑ PED_4_C	299149-20	Convicted Offender			false	

Pedigree Chart

Relationship testing involves testing known genotypes in a familial relationship for simple Mendelian Inheritance laws. Since the core loci selected for forensic genotyping demonstrate the Mendelian characteristics of segregation and independent assortment, the **Pedigree** tool in GeneMarker is an excellent choice to determine genetic relationships between family members.

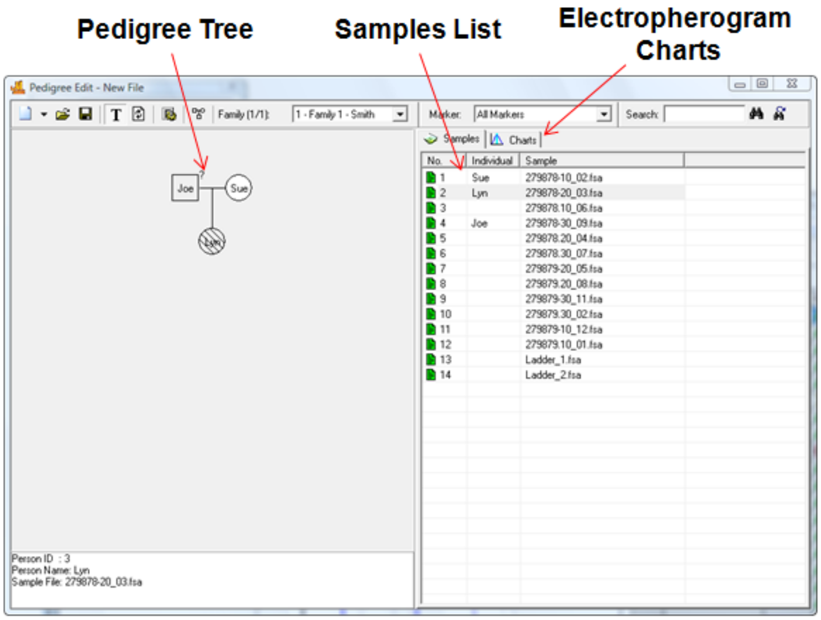
Overview

The *Pedigree* module is designed to aid identification of inheritance patterns and abnormalities. All individuals in the *Pedigree Tree* are directly linked to their corresponding *Electropherograms*. To display the *Electropherogram* for a sample, simply double-click on the sample's node in the *Pedigree Tree* and the *Electropherogram* will appear in the *Charts* tab. The link between the *Pedigree Tree* and *Electropherogram Charts* makes relationship analysis quick and efficient.

To activate the *Pedigree* function, select **Applications → Pedigree** from the *Main Analysis* window menu bar.

NOTE: The dataset must be sized and a Panel applied prior to using the Pedigree function.

Pedigree Application



Family (1/1): 1 - Family 1 - Smith

Marker: All Markers

No.	Individual	Sample
1	Sue	279878-10_02.fsa
2	Lyn	279878-20_03.fsa
3		279878.10_06.fsa
4	Joe	279878-30_09.fsa
5		279878.20_04.fsa
6		279878.30_07.fsa
7		279879-20_05.fsa
8		279879.20_08.fsa
9		279879-30_11.fsa
10		279879.30_02.fsa
11		279879-10_12.fsa
12		279879.10_01.fsa
13		Ladder_1.fsa
14		Ladder_2.fsa

Person ID : 3
Person Name: Lyn
Sample File: 279878-20_03.fsa

Pedigree Tree

The *Pedigree Tree* displays, in a standard format, the relationship between different nodes or individuals in the sample dataset. Older generations appear at the top of the *Pedigree Tree*, younger generations near the bottom. Males are represented as square nodes, females as circle nodes. Horizontal lines indicate mates, vertical lines indicate offspring.

Once a node is added to the *Pedigree Tree*, right-click the node to see additional options.

Select/Deselect Individual Node

To view the electropherogram for an individual, right-click their node and select *Select Node* OR double-click the node and the electropherogram trace will appear in the *Charts* tab on the right. To hide the sample's electropherogram, right-click the node and select *Deselect Node*. When a node is selected in the *Pedigree Tree*, the corresponding sample in the *Sample List* becomes highlighted.

Add/Edit/Delete Individual

If an individual is the first to be added to the *Pedigree Tree*, a family must be designated for the person. If the person is already added to the *Pedigree Tree*, right-click and select *Edit Node* to change that person’s characteristics. To delete an individual from the *Pedigree Tree*, right-click the node and select *Delete Node*.

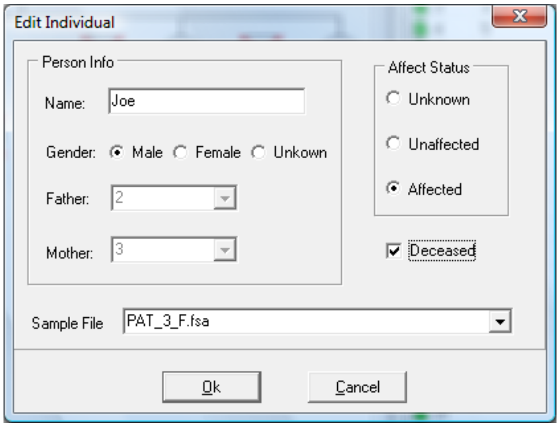
Family Name

Enter a name for the family in the free form text box. The *Family Name* field will not appear after the first individual is added to the *Pedigree Tree*. All subsequent individuals added will be considered members of the family.

Person Info

Name: Free form text box to enter a name for the individual. Display the in dividual’s name in the *Pedigree Tree* by clicking the **Show Individual ID** icon in the toolbar.
Gender: Select either Male, Female, or Unknown gender for the individual. Male nodes are squares, female nodes are circles, and Unknown gender nodes are displayed as a circle within a square.
Father/Mother: When more than one mate is displayed for a Mother or Father, a drop-down menu allows the user to choose which possible parent to associate with the child.

NOTE: The *Gender* and *Father/Mother* options are not available when adding a mate.



Name: Joe
Gender: ● Male ○ Female ○ Unkown
Father: 2
Mother: 3
○ Unknown
○ Unaffected
● Affected
☑ Deceased
Sample File: PAT_3_F.fsa

Affected Status

Affected Status options are available to mark individual nodes for genetic linkage calculations. Before marking individual nodes with *Affected Status*, click the **Pedigree Parameters** icon in the toolbar and adjust settings accordingly.
Unknown: The individual’s phenotype is unknown. The node is displayed as an empty square or circle.
Unaffected: The individual does not show signs of the expected phenotype. The node is filled in white.
Affected: The individual expresses the phenotype. The node is filled in with diagonal hashed lines.

Deceased

If the person is deceased select deceased in the individual edit screen. This extended information – affect status and deceased, is saved and can be imported with the standard pedigree information for future edits in an extended pedigree.

Sample File

Select the individual’s sample file from the drop-down list. Only samples in the current dataset will be available. If no sample file is chosen, the node will be greyed out. Additionally, drag and drop a sample from the *Sample List* onto a node to associate the sample with the individual node.

Add Family Members

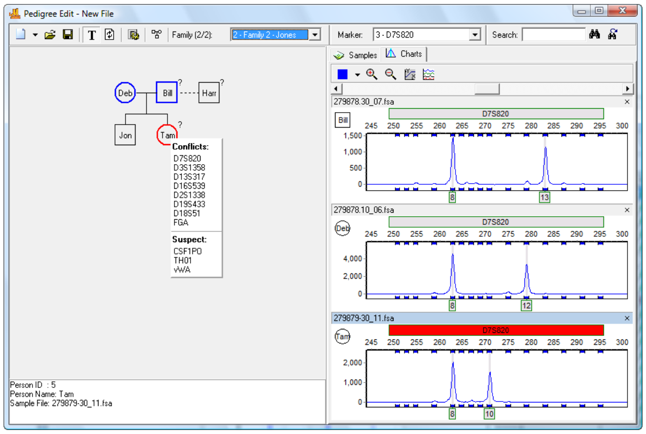
To add family members to the *Pedigree Tree*, right-click a node and select *Add Mate* or *Add Child*. The *Add Mate* or *Add Child* box will appear. Enter the individual’s information and click **OK**.

Display Conflicts

Once a family is created in the *Pedigree Tree*, GeneMarker will automatically identify any Mendelian inheritance conflicts between Parents and Children or between Siblings. Click the **Show Conflict with Parents/Siblings** icon in the toolbar to alternate between the two modes. Nodes with conflicts will appear red. Mouse over the red node and a list of Markers or loci with conflicts will appear. Single left-click the Marker name and the family members’ electropherograms will appear in the *Charts* tab on the right. The conflicting Marker will appear red.

Additionally, suspect Markers are indicated in the Pedigree Tree by a question mark symbol “?” next to the individual’s node. Suspect Markers include those which contain additional peaks or low quality peaks. Mouse over the node with suspect Markers and a list of Markers or loci with suspect peaks will appear. Single left-click the Marker name and the individual's electropherogram will appear in the *Charts* tab on the right. The suspect Marker will appear red.

Show Inheritance Conflicts


Pedigree Edit - New File
Family (2/2):
2 - Family 2 - Jones
Marker:
3 - D7S820
Search:
Samples
Charts
Deb
Bill
Harr
Jon
Tam
279878.30_07.fsa
279878.10_06.fsa
279879-30_11.fsa
D7S820

Conflicts:
D7S820
D3S1358
D13S317
D16S539
D2S1338
D19S433
D18S51
FGA

Suspect:
CSF1PO
TH01
vWA

Person ID : 5
Person Name: Tam
Sample File: 279879-30_11.fsa

Samples List

The *Samples List* displays the filename and individual ID for each sample in the current dataset. Drag and drop samples from the *Sample List* to nodes in the *Pedigree Tree* to associate individuals with the family.

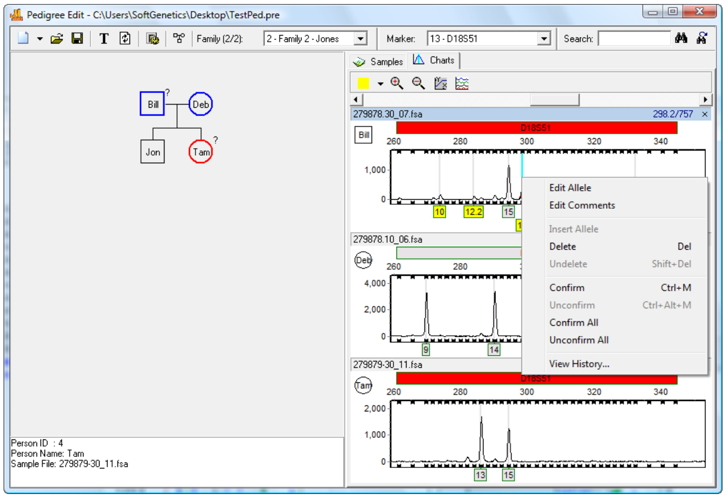
Electropherogram Charts

The electropherogram traces for the samples in the Sample List appear in the Charts tab. Double-click a node or single-click the Marker name in the Conflict or Suspect list of the Pedigree Tree and the electropherogram trace for the Marker will appear. If a Child node is selected, the Mother and Father traces will also appear in the *Charts* tab.

Navigation and allele editing in the *Electropherogram Charts* is similar to navigation options in the *Main Analysis* window. **See Chapter 3 Main Analysis Overview**.

NOTE: After alleles are edited in the *Electropherogram Charts*, click the **Update Sample Data** icon in the toolbar. Conflict and Suspect Marker identification will be adjusted accordingly. Additionally, allele changes made in the *Pedigree* tool will be applied in the *Main Analysis* window.

Edit Suspect Alleles


Pedigree Edit - C:\Users\SoftGenetics\Desktop\TestPed.pre
Family (2/2):
2 - Family 2 - Jones
Marker:
13 - D18S51
Search:
Samples
Charts
Bill
Deb
Jon
Tam
279878.30_07.fsa
298.2/757
D18S51
279878.10_06.fsa
279879-30_11.fsa
Edit Allele
Edit Comments
Insert Allele
Delete Del
Undelete Shift+Del
Confirm Ctrl+M
Unconfirm Ctrl+Alt+M
Confirm All
Unconfirm All
View History...

Person ID : 4
Person Name: Tam
Sample File: 279879-30_11.fsa

Procedure

Although allele calls can be edited in the *Pedigree* tool, it is easier to begin a relationship test analysis with good, clean traces. In order to begin with the best sample traces, complete size calling, Panel alignment, and allele editing in the *Main Analysis* window prior to launching the *Pedigree* tool. To launch the *Pedigree* tool, select *Applications → Pedigree* from the menu bar of the *Main Analysis* window.

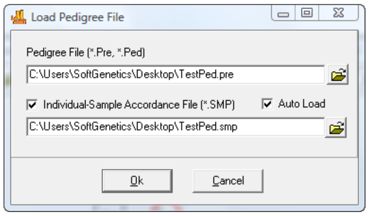
File types accepted or generated by GeneMarker *Pedigree* module:

Pedigree File (PRE, PED) – Contains information about the *Pedigree Tree* display.
Individual Sample Accordance File (SMP) – Contains Family and Individual ID information associated with specific sample file names.
Loci Description File (DAT) – Contains gene frequency information generated in the *Pedigree Parameters* box.

There are two ways to begin a relationship test with the Pedigree tool: upload a previously created pedigree file, OR create an entirely new pedigree file. The procedures are described below.

Upload Previously Created Pedigree

1. In *Pedigree* tool, click the **Open Pedigree File** icon
2. The *Load Pedigree File* box appears
3. Click the **Open Files** icon next to the *Pedigree Files* field
4. Use the Windows Explorer window to locate the Pedigree File (PED, PRE)
5. Select the file and click **Open**



Pedigree File (*.Pre, *.Ped)
C:\Users\SoftGenetics\Desktop\TestPed.pre
☑ Individual-Sample Accordance File (*.SMP) ☑ Auto Load
C:\Users\SoftGenetics\Desktop\TestPed.smp

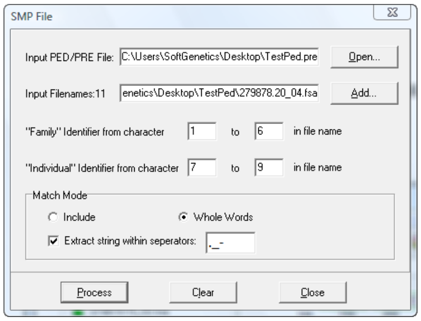
NOTE: The *Pedigree File* selected must be associated with the samples currently uploaded to GeneMarker. If the sample filenames are not the same, the *Pedigree Tree* will appear grey and will not link with the *Sample List* or *Electropherogram Chart*.

6. The directory path will appear in the *Pedigree File* field
7. The same directory path and filename will appear in the *Individual Sample Accordance File* field if the SMP file is located in the same folder
8. If the SMP file is located in a different directory, click the **Open Files** icon next to the *Individual Sample Accordance File* field, select the correct directory, and click **Open**
9. Click **OK** in the *Load Pedigree File* box
10. The information in the Pedigree and SMP files will recreate the *Pedigree Tree* and associate the nodes with the samples in the dataset.

Create an SMP File from a PED/PRE File

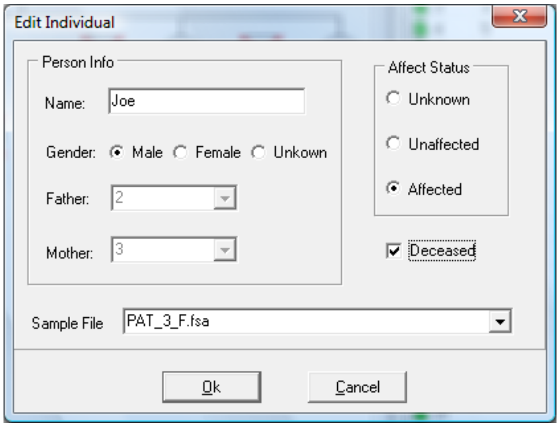
If an SMP file does not exist for a *Pedigree File* or has been lost, a new SMP file can be generated with the *Pedigree File Name Match* tool.

1. In the *Main Analysis* window menu bar, select *Tools → Pedigree File Name Match*
2. The *SMP File* box appears
3. Click the **Open** button
4. Select a previously created Pedigree File (PED or PRE)
5. Click the **Add** button
6. Select all the samples included in the Pedigree File
7. Click the **Process** button
8. The *SMP Table* box will appear with filenames aligned in family groups
9. Click the **Save As** button
10. Save the SMP file to the same directory that contains the Pedigree File
11. Proceed through steps **1-10** above.



Input PED/PRE File: C:\Users\SoftGenetics\Desktop\TestPed.pre
Input Filenames:11 enetics\Desktop\TestPed\279878.20_04.fsa
"Family" Identifier from character 1 to 6 in file name
"Individual" Identifier from character 7 to 9 in file name
Match Mode
○ Include ● Whole Words
☑ Extract string within seperators: ._-

Create New Pedigree


Edit Individual
Person Info
Affect Status
○ Unknown
○ Unaffected
● Affected
Ok
Cancel

Name: Joe

Gender: ● Male ○ Female ○ Unkown

Father: 2

Mother: 3

☑ Deceased

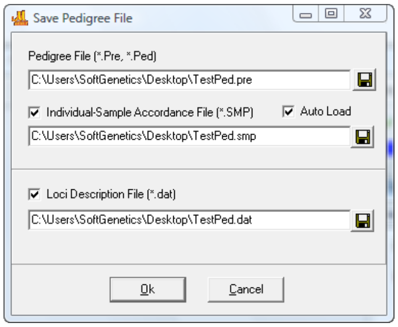
Sample File: PAT_3_F.fsa

1. In *Pedigree* tool, click the **New Pedigree File** icon
2. The *New Family (New Individual)* box appears
3. Enter a Family Name, the Individual's Name, Gender, and the Sample File to associate with the Pedigree Tree node
4. Click **OK**
5. The individual's node will appear in the *Pedigree Tree* and the individual ID information will appear next to the sample name in the *Sample List*.
6. Continue to add family members by right-clicking at the node and selecting *Add Mate/Child*
7. Mouse over red highlighted nodes in the *Pedigree Tree* to view Conflicts and Suspect Markers – see section above – *Pedigree Tree*
8. Edit alleles in the *Electropherogram Charts* as required – see section above – *Electropherogram Charts*

Save Pedigree File

After the Pedigree has been created and modified, there are two options for saving the information: save the Pedigree File and/or export the *Pedigree Tree* as an image.

Save Pedigree File


Save Pedigree File
Ok
Cancel

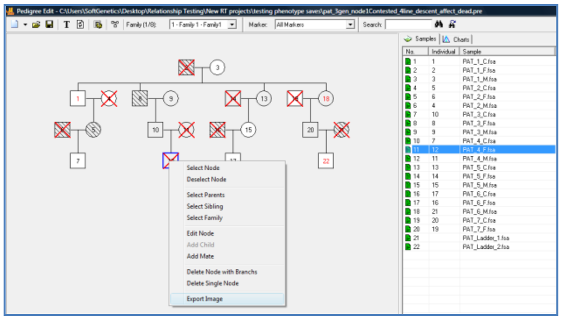
Pedigree File (*.Pre, *.Ped)
C:\Users\SoftGenetics\Desktop\TestPed.pre

☑ Individual-Sample Accordance File (*.SMP) ☑ Auto Load
C:\Users\SoftGenetics\Desktop\TestPed.smp

☑ Loci Description File (*.dat)
C:\Users\SoftGenetics\Desktop\TestPed.dat

1. In *Pedigree* tool, click the **Save Pedigree File** icon
2. The *Save Pedigree File* box appears
3. Click the **Save** icon next to the *Pedigree File* field
4. The Windows Explorer directory window appears
5. Enter a filename and select a directory
6. Click **Save**
7. The directory path and filename will appear in all three fields of the *Save Pedigree File* box
8. Click **OK**
9. Three files will be saved to the specified directory (PRE, SMP, and DAT).
10. To reopen the files in *Pedigree* tool, follow steps **1-10** of the *Upload Previously Created Pedigree* section above

Save Pedigree Tree


Pedigree Edit - C:\Users\SoftGenetics\Desktop\Relationship Testing\New RT projects\testing phenotype saves\pat_3gen_node1Contested_4line_descent_affect_dead.pre
Samples
Charts
3
1
9
13
18
10
15
20
7
17
22
Select Node
Deselect Node
Select Parents
Select Sibling
Select Family
Edit Node
Add Child
Add Mate
Delete Node with Branchs
Delete Single Node
Export Image

Family (1/8): 1 - Family 1 - Family1

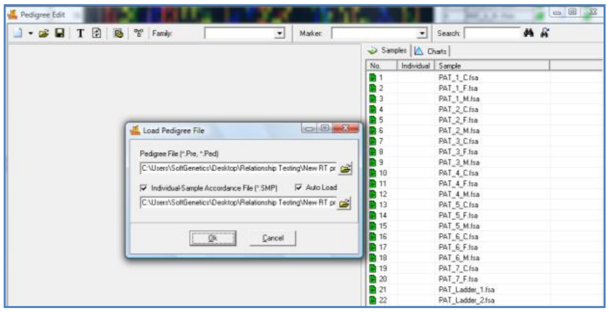
Marker: All Markers

Search:

No.	Individual	Sample
1	1	PAT_1_C.fsa
2	2	PAT_1_F.fsa
3	3	PAT_1_M.fsa
4	5	PAT_2_C.fsa
5	6	PAT_2_F.fsa
6	4	PAT_2_M.fsa
7	10	PAT_3_C.fsa
8	8	PAT_3_F.fsa
9	9	PAT_3_M.fsa
10	7	PAT_4_C.fsa
11	12	PAT_4_F.fsa
12	11	PAT_4_M.fsa
13	13	PAT_5_C.fsa
14	14	PAT_5_F.fsa
15	15	PAT_5_M.fsa
16	17	PAT_6_C.fsa
17	16	PAT_6_F.fsa
18	21	PAT_6_M.fsa
19	20	PAT_7_C.fsa
20	19	PAT_7_F.fsa
21		PAT_Ladder_1.fsa
22		PAT_Ladder_2.fsa

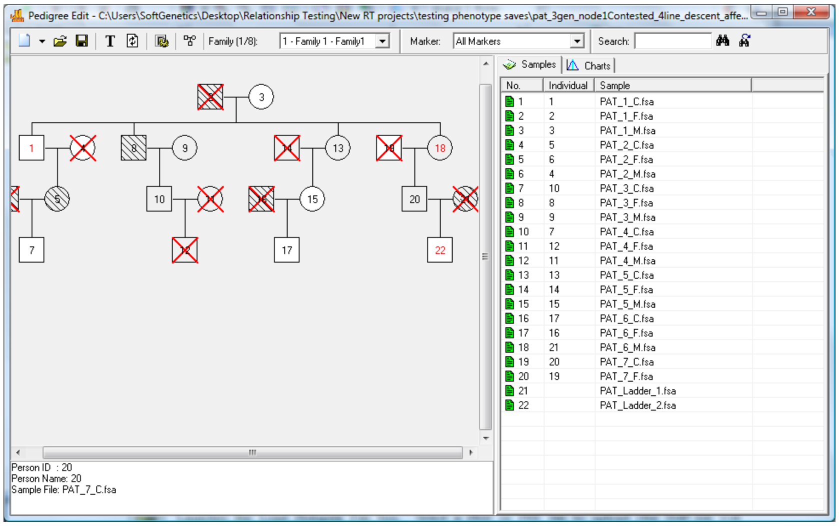
1. Right-click anywhere in the *Pedigree Tree*
2. Select **Export Bitmap**
3. The *Save As* window appears
4. Enter a filename, choose a directory, and click **Save**
5. The *Pedigree Tree* will be saved as an image file (BMP)

Extended Pedigree Files
All personal information is saved and retrieved with the pedigree file, including affected status and deceased. Extended family trees can be built saved and added to, as additional information becomes available.



Pedigree File (*.Pre, *.Ped)
C:\Users\SoftGenetics\Desktop\Relationship Testing\New RT pr

☑ Individual-Sample Accordance File (*.SMP) ☑ Auto Load
C:\Users\SoftGenetics\Desktop\Relationship Testing\New RT pr



Pedigree Edit - C:\Users\SoftGenetics\Desktop\Relationship Testing\New RT projects\testing phenotype saves\pat_3gen_node1Contested_4line_descent_affe...

Family (1/8): 1 - Family 1 - Family1 Marker: All Markers Search:

No.	Individual	Sample
1	1	PAT_1_C.fsa
2	2	PAT_1_F.fsa
3	3	PAT_1_M.fsa
4	5	PAT_2_C.fsa
5	6	PAT_2_F.fsa
6	4	PAT_2_M.fsa
7	10	PAT_3_C.fsa
8	8	PAT_3_F.fsa
9	9	PAT_3_M.fsa
10	7	PAT_4_C.fsa
11	12	PAT_4_F.fsa
12	11	PAT_4_M.fsa
13	13	PAT_5_C.fsa
14	14	PAT_5_F.fsa
15	15	PAT_5_M.fsa
16	17	PAT_6_C.fsa
17	16	PAT_6_F.fsa
18	21	PAT_6_M.fsa
19	20	PAT_7_C.fsa
20	19	PAT_7_F.fsa
21		PAT_Ladder_1.fsa
22		PAT_Ladder_2.fsa

Person ID : 20
Person Name: 20
Sample File: PAT_7_C.fsa

Icons and Functions

New Pedigree File
Select *New Pedigree File* to create a new pedigree with multiple families OR select *New Family* to add a family to the pedigree file. Enter the first family member's information into the *New Family (New Individual)* box and click **OK** to create a new *Pedigree Tree*.

Open Pedigree File
Launches the Load Pedigree File box. Select a PED or PRE file to upload (the SMP file will automatically upload) and click OK.

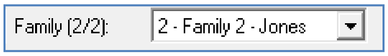
Save Pedigree File
Launches the *Save Pedigree File* box. Enter filename and change directory to save the Pedigree Files (PRE, SMP, DAT).

Show Individual Name
When selected, the individual ID will be displayed in the nodes of the *Pedigree Tree*.

Update Sample Data
Select to refresh the Mendelian inheritance calculation after a node or allele is edited.

Pedigree Parameters
Launches the *Loci Description* box. Enter the Affection Locus Description, Gene Frequencies, Select Markers, Number of Liability Classes, Penetrances, Recombination Values, and view the Allele Label and Frequencies.

Show Conflict
Toggle between *Show Conflict with Parents* and *Show Conflict with Sibling*. Conflicting and suspected Markers based on Mendelian inheritance are highlighted.



Family (2/2): 2 - Family 2 - Jones

Family
Select a family from the currently uploaded pedigree file to view and edit.

Marker: 12 - TPOX

Marker
Select a Marker or Locus to view in the *Electropherogram Charts*.

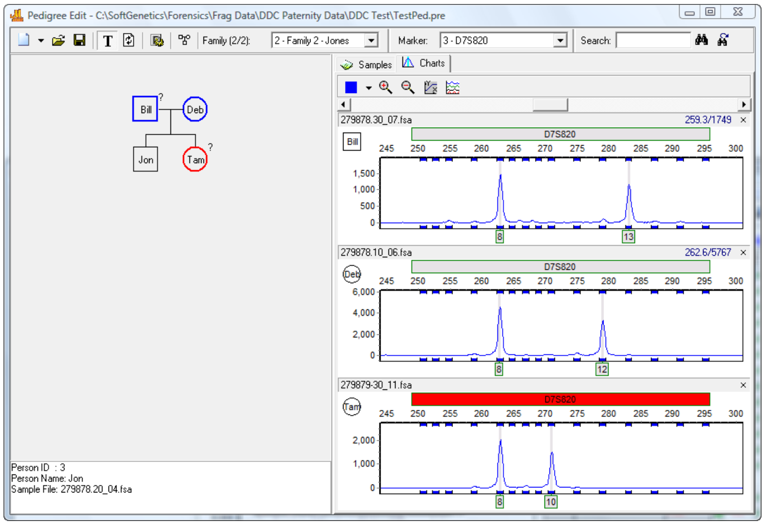
What to Expect

Mendelian inheritance is guided by Mendel's two basic laws: Segregation and Independent Assortment. The law of segregation describes how phenotypes are an expression of two alleles inherited independently, one from each parent. Independent assortment is the idea that the inheritance of one gene or allele does not affect the likelihood of inheritance of a different gene. Today we have found that this is not true of all genes, some genes are invariably linked and inherited together. Linked genes do not follow Mendelian inheritance patterns.

With GeneMarker's Pedigree module, Mendelian inheritance patterns can be analyzed. That is, alleles are inherited independently, one from each parent, and alleles are not influence by the inheritance of other alleles. Forensic STR loci were selected because they follow the laws of Mendelian genetics.

In the example below, the child's Marker D7S820 is highlighted red. The child's Allele 8 matches with the mother and father; however, the child's Allele 10 does not match with either the father or the mother. Because Allele 10 does not match an allele from the father or mother, it can be assumed that one of the parents is not related to the child. Because all three individuals share Allele 8, it cannot be determined which parent is not related to the child. Review additional loci to determine which parent is unrelated.

Mendelian Inheritance of Alleles



Pedigree Edit - C:\SoftGenetics\Forensics\Frag Data\DDC Paternity Data\DDC Test\TestPed.pre

Family (2/2): 2 - Family 2 - Jones

Marker: 3 - D7S820

Search:

Person ID : 3
Person Name: Jon
Sample File: 279878.20_04.fsa

Kinship Analysis

Overview

Identity by descent (IBD) uses the number of alleles shared and allele frequencies to calculate the probability that two individuals have a specific relationship versus the probability that two random individuals from that population have the given genotypes. IBD excludes any individuals that are not potential relatives and allows ranking of potential relatives by probability of relationship level. STR profiles of two individuals can be compared to determine the likelihood that they have a specific relationship versus the likelihood that they are unrelated. Kinship analysis compares STR profiles from individuals to determine likelihood of a family relationship versus the likelihood that two individuals with these STR profiles are unrelated. The formulas used to calculate the level of kinship depend on:

1. Probabilities that 2, 1 or 0 alleles will be shared (IBD identity by descent) given a specific relationship

2. The probability of a specific genotype X given genotype Y at all loci, under the conditions that X and Y have 2, 1, or 0 alleles IBD

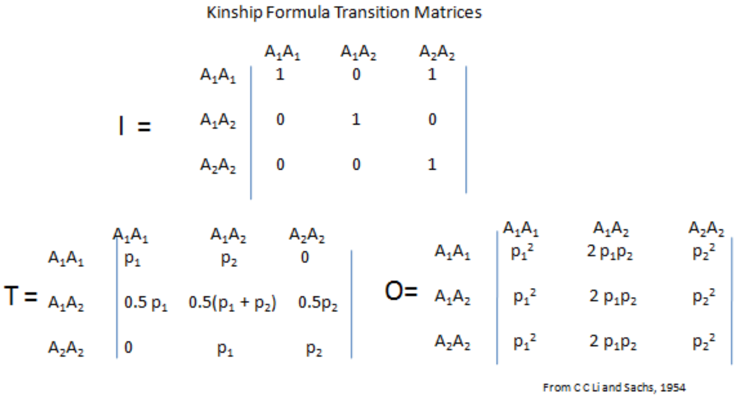
GeneMarker uses established, rigorous statistical analysis (Kinship formulas from Brenner 2004; Eisenberg and Planz 2007) to calculate probabilities and likelihood ratios for different relationship levels including: Parent/child, Siblings, Half-siblings, Uncle/Nephew, Cousins and Grandparents.

$$[P_{2(xy)}\,\Phi_2] + [P_{1(xy)}\,\Phi_1] + [P_{0(xy)}\,\Phi_0] \qquad \text{(eq. 1)}$$

Where:
$P_{2(xy)}$ = Probability of 2 alleles IBD (I) given the genotypes of sample x and sample y
$P_{1(xy)}$ = Probability of 1 alleles IBD (T) given the genotypes of sample x and sample y
$P_{0(xy)}$ = Probability of 0 alleles IBD (O) given the genotypes of sample x and sample

Identity by Descent (IBD Φ_2, Φ_1, Φ_0) for each relationship category:

	Φ_2	Φ_1	Φ_0
Parent – Child	0	1	0
Siblings	0.25	0.5	0.25
Half-siblings	0	0.5	0.5
Cousins	0	o.25	0.75
Uncle/nephew	0	0.5	0.5
Grandparent/ Grandchild	0	0.5	0.5



Kinship Formula Transition Matrices

$$I = \begin{array}{c|ccc|} & A_1A_1 & A_1A_2 & A_2A_2 \\ A_1A_1 & 1 & 0 & 1 \\ A_1A_2 & 0 & 1 & 0 \\ A_2A_2 & 0 & 0 & 1 \end{array}$$

$$T = \begin{array}{c|ccc|} & A_1A_1 & A_1A_2 & A_2A_2 \\ A_1A_1 & p_1 & p_2 & 0 \\ A_1A_2 & 0.5\,p_1 & 0.5(p_1 + p_2) & 0.5p_2 \\ A_2A_2 & 0 & p_1 & p_2 \end{array} \qquad O = \begin{array}{c|ccc|} & A_1A_1 & A_1A_2 & A_2A_2 \\ A_1A_1 & p_1^2 & 2\,p_1p_2 & p_2^2 \\ A_1A_2 & p_1^2 & 2\,p_1p_2 & p_2^2 \\ A_2A_2 & p_1^2 & 2\,p_1p_2 & p_2^2 \end{array}$$

From C C Li and Sachs, 1954

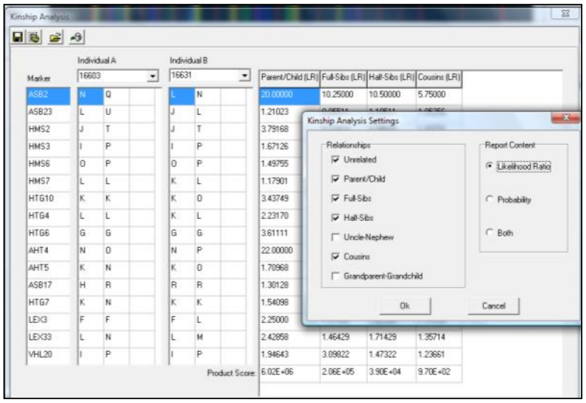
All derivations of the Kinship equation 1
can be located at http://www.softgenetics.com/images/formulas.jpg

Procedure

Although allele calls can be edited in the *Relationship Testing* tool, it is easier to begin a relationship test analysis with good, clean traces. In order to begin with the best sample traces, complete size calling, Panel alignment, and allele editing in the *Main Analysis* (see chapter 2 General Procedure) window prior to launching the *Relationship Testing* tool.
File types accepted or generated by GeneMarker *Relationship Testinge* module:
Pre/Ped files, SGF, TXT, BMP, JPG, PNG, CMF

The *Save to DataBase* function allows easy updates of the relationship testing database directly from the genotyping results of GeneMarker. Additionaly, previously genotyped files can be submitted to the database when saved in a .cmf file – Select *Applications* →*Relationship Testing* → *DataBase* → *save to database* → *load from CMF*
To launch the *Kinship Analysis* function, select *Applications* → *Relationship Testing* from the menu bar of the *Main Analysis* window.

1. Open data file or previously saved project
2. Run Wizard to call alleles
3. Select Relationship Testing from the Applications drop down menu
4. Select Allele Frequency of the appropriate population from the Tools drop down menu. The dropdown menu contains all samples from the current project. Additional samples can be added to the dropdown list by using the icons to open a folder or select files from the database.
5. Select Kinship Analysis Tool – use drop down menus to select individuals for comparison



Individual A: 16603
Individual B: 16631

Marker	Individual A			Individual B			Parent/Child (LR)	Full-Sibs (LR)	Half-Sibs (LR)	Cousins (LR)
ASB2	N	Q		L	N		20.00000	10.25000	10.50000	5.75000
ASB23	L	U		J	L		1.21023			
HMS2	J	T		J	T		3.79168			
HMS3	I	P		I	P		1.67126			
HMS6	O	P		O	P		1.49755			
HMS7	L	L		K	L		1.17901			
HTG10	K	K		K	O		3.43749			
HTG4	L	L		K	L		2.23170			
HTG6	G	G		G	G		3.61111			
AHT4	N	O		N	P		22.00000			
AHT5	K	N		K	O		1.70968			
ASB17	H	R		R	R		1.30128			
HTG7	K	N		K	K		1.54098			
LEX3	F	F		F	L		2.25000			
LEX33	L	N		L	M		2.42858	1.46429	1.71429	1.35714
VHL20	I	P		I	P		1.94643	3.09822	1.47322	1.23661
						Product Score:	6.02E+06	2.06E+05	3.90E+04	9.70E+02

Relationships
☑ Unrelated
☑ Parent/Child
☑ Full-Sibs
☑ Half-Sibs
☐ Uncle-Nephew
☑ Cousins
☐ Grandparent-Grandchild

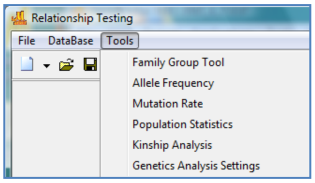
Report Content
● Likelihood Ratio
○ Probability
○ Both

6. Select the desired relationship level and likelihood ratio, probability or both at the kinship analysis settings
7. Probabilities for the occurrence of the genotypes within the population having a specific relationship or being unrelated for each locus and all loci combined are displayed in table form
8. Likelihood ratios for each locus and combined likelihood ratio of a related (parent/child, sibling, half sibling) are presented in table form.

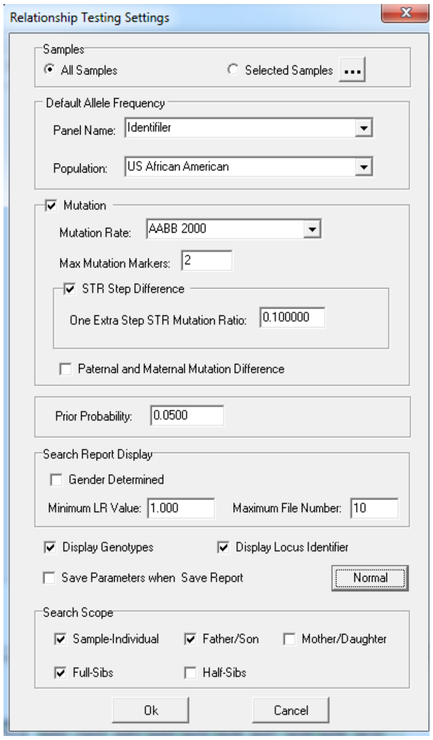
Icons and Functions

Relationship Testing
File
DataBase
Tools

Relationship Testing Main Drop-down menus
Select from File, DataBase or Tool options



Relationship Testing Tools include:
Family Group Tool – for automated pedigree trio drawing
Allele Frequency Import species specific allele frequency .txt files Tools →Allele Frequency → open folder icon →select file → save
Mutation Rate Import species specific .txt file
Population Statistics for the file under analysis



Samples
● All Samples ○ Selected Samples

Default Allele Frequency
Panel Name: Identifiler
Population: US African American

☑ Mutation
Mutation Rate: AABB 2000
Max Mutation Markers: 2
☑ STR Step Difference
One Extra Step STR Mutation Ratio: 0.100000
☐ Paternal and Maternal Mutation Difference

Prior Probability: 0.0500

Search Report Display
☐ Gender Determined
Minimum LR Value: 1.000 Maximum File Number: 10

☑ Display Genotypes ☑ Display Locus Identifier
☐ Save Parameters when Save Report

Search Scope
☑ Sample-Individual ☑ Father/Son ☐ Mother/Daughter
☑ Full-Sibs ☐ Half-Sibs

Genetic Analysis Settings – allows setting the above options at the same time

Tolerate mutations or genotyping error (in wild life genotypes) by setting the maximum mutation markers. When this is selected the program will substitute the mutation rate/mean PE for the kinship equation at that locus. The resulting probability and likelihood ratio will be much lower, but not zero, as they would be if the mutation tolerance is de-selected.

Search Report Display – If gender information is present in the genotype select Gender determined to display Father/Son and Mother/Daughter potential relationships. If gender information is not present, as in the case of monoecious plants and many invertebrates, de-select Gender Determined. All files with a potential parent/child relationship will be displayed. Limit the number of positive files retrieved by minimum likelihood ratio value or maximum file number. Advanced settings should be used to select the relationship levels reported for database searching. Default setting will display most likely same individual, father/son, mother/daughter, sibling and half-siblings. When multiple files are located for a specific kinship level, they are ranked by likelihood ratio (LR).

New Pedigree File

Select *New Pedigree File* to create a new pedigree with multiple families *OR* select *New Family* to add a family to the pedigree file. Enter the first family member's information into the *New Family (New Individual)* box and click **OK** to create a new *Pedigree Tree*.

Open Pedigree File

Launches the Load Pedigree File box. Select a PED or PRE file to upload (the SMP file will automatically upload) and click OK.

Save Pedigree File

Launches the *Save Pedigree File* box. Enter filename and change directory to save the Pedigree Files (PRE, SMP, DAT).

Show Individual Name

When selected, the individual ID will be displayed in the nodes of the *Pedigree Tree*.

Update Sample Data

Select to refresh the Mendelian inheritance calculation after a node or allele is edited and after selection a different family when the *'show genotype'* display is used

Relationship Testing Parameters

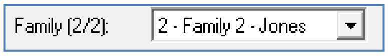
Launches the *Relationship Testing Settings* box. Options for selected samples or all samples, selecting the appropriate allele frequency for the population, mutation rate, and prior probabilitiy.

Show Conflict

Toggle between *Show Conflict with Parents* and *Show Conflict with Sibling*. Conflicting and suspected Markers based on Mendelian inheritance are highlighted.

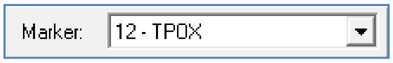
Show Genotype

Toggle between *Displaying and Not Displaying the Genotypes* of the selected node



Family (2/2): 2 - Family 2 - Jones

Family
Select a family from the currently uploaded pedigree file to view and edit.



Marker: 12 - TPOX

Marker
Select a Marker or Locus to view in the *Electropherogram Charts*.

Show Color
Allows the user to select all colors to view, hide all colors, or choose a single dye layer. Choose a single dye by single left mouse clicking on the icon.

Zoom In
Use the icon to zoom in on the image, or hold down the left mouse button and draw a box, from the top left corner to bottom right corner, around the area you wish to zoom in.

Zoom Out
Use the icon to zoom out on the image, or hold down the left mouse button and draw a box, from the bottom right corner to top left corner.

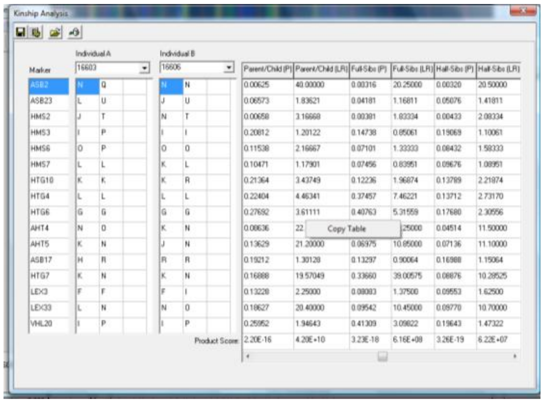
Set Axis
The default setting automatically sets the Y-axis according to the maximum peak intensity of the samples. Two other options are available: auto fit the Y-axis using peak intensities of the alleles, or the user can select the ranges for the X- and Y-axis.

Browse by All Colors
Displays a comparative view of sample electropherograms by dye color. Individual samples can be selected from the drop-down menu.

Save and Print Report

Kinship analysis - Save tables for export in .txt files

Or right click on the table to copy/ paste into an existing document or report



Marker	Individual A 16603		Individual B 16606		Parent/Child (P)	Parent/Child (LR)	Full-Sibs (P)	Full-Sibs (LR)	Half-Sibs (P)	Half-Sibs (LR)
ASB2	N	Q	N	N	0.00625	40.00000	0.00316	20.25000	0.00320	20.50000
ASB23	L	U	J	U	0.06573	1.83621	0.04181	1.16811	0.05076	1.41811
HMS2	J	T	N	T	0.00658	3.16668	0.00381	1.83334	0.00433	2.08334
HMS3	I	P	I	I	0.20812	1.20122	0.14738	0.85061	0.19069	1.10061
HMS6	O	P	O	O	0.11538	2.16667	0.07101	1.33333	0.08432	1.58333
HMS7	L	L	K	L	0.10471	1.17901	0.07456	0.83951	0.09676	1.08951
HTG10	K	K	K	R	0.21364	3.43749	0.12236	1.96874	0.13789	2.21874
HTG4	L	L	L	L	0.22404	4.46341	0.37457	7.46221	0.13712	2.73170
HTG6	G	G	G	G	0.27692	3.61111	0.40763	5.31559	0.17680	2.30556
AHT4	N	O	K	N	0.08636	22		25000	0.04514	11.50000
AHT5	K	N	J	N	0.13629	21.20000	0.06975	10.85000	0.07136	11.10000
ASB17	H	R	R	R	0.19212	1.30128	0.13297	0.90064	0.16988	1.15064
HTG7	K	N	K	N	0.16888	19.57049	0.33660	39.00575	0.08876	10.28525
LEX3	F	F	F	I	0.13228	2.25000	0.08083	1.37500	0.09553	1.62500
LEX33	L	N	N	O	0.18627	20.40000	0.09542	10.45000	0.09770	10.70000
VHL20	I	P	I	P	0.25952	1.94643	0.41309	3.09822	0.19643	1.47322
Product Score					2.20E-16	4.20E+10	3.23E-18	6.16E+08	3.26E-19	6.22E+07

Importing Species Specific Allele Frequency and Mutation Rates

It is very easy to import region-specific or species specific values for use in the kinship calculations. Population specific allele frequency and mutation rate tables must follow the format of preloaded files and be saved as a .txt tab delimited file. Export one of the preloaded allele frequency tables and use it as a template to form an allele frequency table for the desired population. Import the new tables by opening the Tools > Allele Frequency > Open folder icon > Select file(s) > Save

Database Search: Locate Duplicate Samples and Nearest Relatives

Overview

The database function is capable of closed system searches for same sample match, identification of nearest relatives and calculating kinship statistics The *Save to DataBase* function allows easy updates of the relationship testing database.

The Data base searching tool is ideal for applications such as:

- Population diversity/inbreeding wild populations
- Controlling rate of inbreeding – wild life management and livestock
- Quantifying natural population size
- Identification of clones within a population
- Identifying and ranking potential relatives
- Verification of the number of successfully breeding individuals
- Identification of lost/ stolen pets or livestock

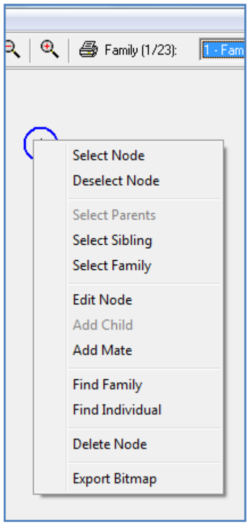
Procedure

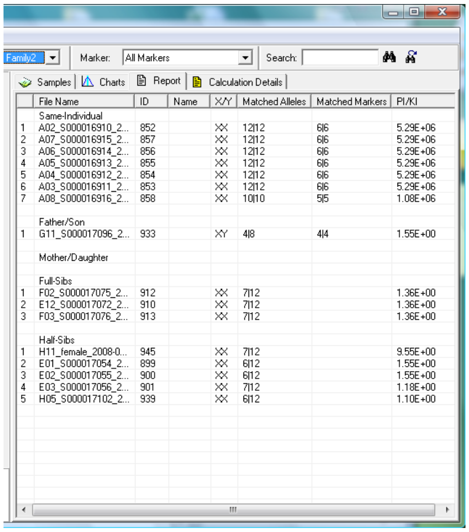
1. Open data file or previously saved project
2. Run Wizard to call alleles
3. Select Relationship Testing from the Applications drop down menu
4. Select Allele Frequency of the appropriate population from the Tools drop down menu

Individual sample

1. Tools > *family group tool* > *OK* to allow selection of each individual in the file as a separate node
2. Use the Family dropdown menu to select the individual file
3. Right click on the node and select *find family* from the drop-down menu
4. Left click on the Report icon in the tool bar to display the file name and any duplicates of that file found in the data base. The samples with the highest LR for each relationship type are displayed in descending order, in addition to the sex, number of matched alleles and matched markers

In this example there are seven samples with the identical microsatellite profile. The random match probability indicates that there is a 1 in 529,000 chance that a different individual in this animal population has the same profile. It is very likely that these are all replicate samples from the same individual.





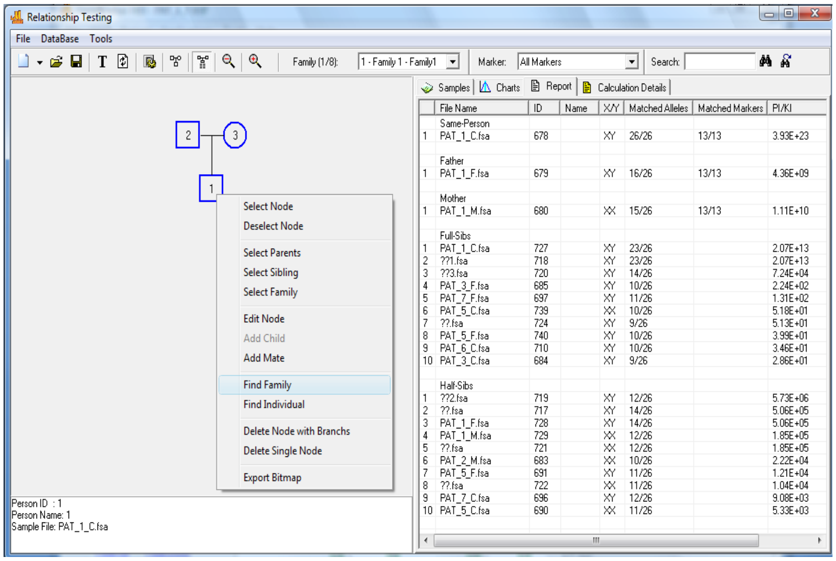
Marker: All Markers

Search:

	File Name	ID	Name	X/Y	Matched Alleles	Matched Markers	PI/KI
	Same-Individual						
1	A02_S000016910_2...	852		XX	12|12	6|6	5.29E+06
2	A07_S000016915_2...	857		XX	12|12	6|6	5.29E+06
3	A06_S000016914_2...	856		XX	12|12	6|6	5.29E+06
4	A05_S000016913_2...	855		XX	12|12	6|6	5.29E+06
5	A04_S000016912_2...	854		XX	12|12	6|6	5.29E+06
6	A03_S000016911_2...	853		XX	12|12	6|6	5.29E+06
7	A08_S000016916_2...	858		XX	10|10	5|5	1.08E+06
	Father/Son						
1	G11_S000017096_2...	933		XY	4|8	4|4	1.55E+00
	Mother/Daughter						
	Full-Sibs						
1	F02_S000017075_2...	912		XX	7|12		1.36E+00
2	E12_S000017072_2...	910		XX	7|12		1.36E+00
3	F03_S000017076_2...	913		XX	7|12		1.36E+00
	Half-Sibs						
1	H11_female_2008-0...	945		XX	7|12		9.55E+00
2	E01_S000017054_2...	899		XX	6|12		1.55E+00
3	E02_S000017055_2...	900		XX	6|12		1.55E+00
4	E03_S000017056_2...	901		XX	7|12		1.18E+00
5	H05_S000017102_2...	939		XX	6|12		1.10E+00

Sample in Pedigree Tree

1. Import or draw Pedigree Tree **See Chapter 7 Pedigree Analysis and Automated Pedigree Tree.**
2. Right click on the node and select *find family* from the drop-down menu
3. Left click on the Report icon in the tool bar to display the file name and any duplicates of that file found in the data base. The samples with the highest LR for each relationship type are displayed in descending order, in addition to the sex, number of matched alleles and matched markers


Relationship Testing
File DataBase Tools
Family (1/8):
1 - Family 1 - Family1
Marker:
All Markers
Search:
Samples
Charts
Report
Calculation Details
2
3
1
Select Node
Deselect Node
Select Parents
Select Sibling
Select Family
Edit Node
Add Child
Add Mate
Find Family
Find Individual
Delete Node with Branchs
Delete Single Node
Export Bitmap

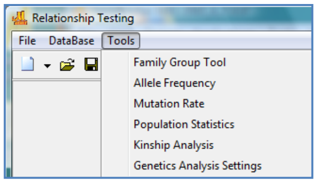
Person ID : 1
Person Name: 1
Sample File: PAT_1_C.fsa

	File Name	ID	Name	X/Y	Matched Alleles	Matched Markers	PI/KI
	Same-Person						
1	PAT_1_C.fsa	678		XY	26/26	13/13	3.93E+23
	Father						
1	PAT_1_F.fsa	679		XY	16/26	13/13	4.36E+09
	Mother						
1	PAT_1_M.fsa	680		XX	15/26	13/13	1.11E+10
	Full-Sibs						
1	PAT_1_C.fsa	727		XY	23/26		2.07E+13
2	??1.fsa	718		XY	23/26		2.07E+13
3	??3.fsa	720		XY	14/26		7.24E+04
4	PAT_3_F.fsa	685		XY	10/26		2.24E+02
5	PAT_7_F.fsa	697		XY	11/26		1.31E+02
6	PAT_5_C.fsa	739		XX	10/26		5.18E+01
7	??.fsa	724		XY	9/26		5.13E+01
8	PAT_5_F.fsa	740		XY	10/26		3.99E+01
9	PAT_6_C.fsa	710		XY	10/26		3.46E+01
10	PAT_3_C.fsa	684		XY	9/26		2.86E+01
	Half-Sibs						
1	??2.fsa	719		XY	12/26		5.73E+06
2	??.fsa	717		XY	14/26		5.06E+05
3	PAT_1_F.fsa	728		XY	14/26		5.06E+05
4	PAT_1_M.fsa	729		XX	12/26		1.85E+05
5	??.fsa	721		XX	12/26		1.85E+05
6	PAT_2_M.fsa	683		XX	10/26		2.22E+04
7	PAT_5_F.fsa	691		XY	11/26		1.21E+04
8	??.fsa	722		XX	11/26		1.04E+04
9	PAT_7_C.fsa	696		XY	12/26		9.08E+03
10	PAT_5_C.fsa	690		XX	11/26		5.33E+03

Icons and Functions

Relationship Testing
File DataBase Tools

Relationship Testing Main Drop-down menus
Select from File, DataBase or Tool options


Relationship Testing
File DataBase Tools
Family Group Tool
Allele Frequency
Mutation Rate
Population Statistics
Kinship Analysis
Genetics Analysis Settings

Relationship Testing Tools include:
Family Group Tool – for automated pedigree trio drawing
Allele Frequency Import species specific allele frequency .txt files
Mutation Rate Import species specific .txt file
Population Statistics for the file under analysis
Genetic Analysis Settings – allows setting the above options at the same time

New Pedigree File
Select *New Pedigree File* to create a new pedigree with multiple families OR select *New Family* to add a family to the pedigree file. Enter the first family member's information into the *New Family (New Individual)* box and click **OK** to create a new *Pedigree Tree*.

Open Pedigree File
Launches the Load Pedigree File box. Select a PED or PRE file to upload (the SMP file will automatically upload) and click OK.

Save Pedigree File
Launches the *Save Pedigree File* box. Enter filename and change directory to save the Pedigree Files (PRE, SMP, DAT).

Show Individual Name
When selected, the individual ID will be displayed in the nodes of the *Pedigree Tree*.

Update Sample Data
Select to refresh the Mendelian inheritance calculation after a node or allele is edited and after selection a different family when the *'show genotype'* display is used

Relationship Testing Parameters
Launches the *Relationship Testing Settings* box. Options for selected samples or all samples, selecting the appropriate allele frequency for the population, mutation rate, and prior probabilitiy.

Show Conflict
Toggle between *Show Conflict with Parents* and *Show Conflict with Sibling*. Conflicting and suspected Markers based on Mendelian inheritance are highlighted.

Show Genotype
Toggle between *Displaying and Not Displaying the Genotypes* of the selected node

Family (2/2): 2 - Family 2 - Jones

Family
Select a family from the currently uploaded pedigree file to view and edit.

Marker: 12 - TPOX

Marker
Select a Marker or Locus to view in the *Electropherogram Charts*.

Show Color
Allows the user to select all colors to view, hide all colors, or choose a single dye layer. Choose a single dye by single left mouse clicking on the icon.

Zoom In
Use the icon to zoom in on the image, or hold down the left mouse button and draw a box, from the top left corner to bottom right corner, around the area you wish to zoom in.

Zoom Out
Use the icon to zoom out on the image, or hold down the left mouse button and draw a box, from the bottom right corner to top left corner.

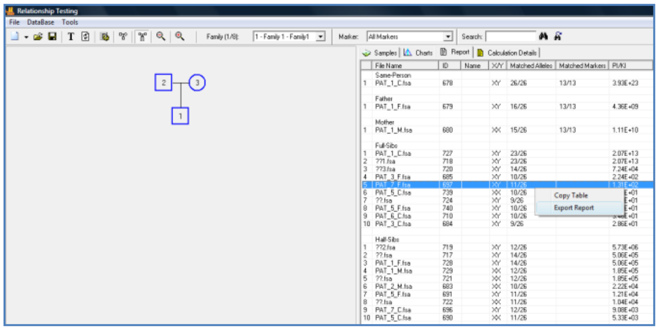
Set Axis
The default setting automatically sets the Y-axis according to the maximum peak intensity of the samples. Two other options are available: auto fit the Y-axis using peak intensities of the alleles, or the user can select the ranges for the X- and Y-axis.

Browse by All Colors
Displays a comparative view of sample electropherograms by dye color. Individual samples can be selected from the drop-down menu.

Save and Print Report

'Find Family' Report – right click on the report to copy/paste directly into an existing document or report

Or export as a .txt file



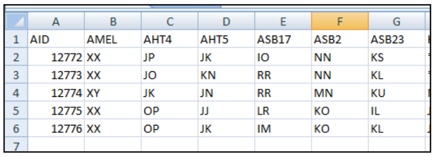
	File Name	ID	Name	X/Y	Matched Alleles	Matched Markers	PI/KI
	Same-Person						
1	PAT_1_C.fsa	678		XY	26/26	13/13	3.93E+23
	Father						
1	PAT_1_F.fsa	679		XY	16/26	13/13	4.36E+09
	Mother						
1	PAT_1_M.fsa	680		XX	15/26	13/13	1.11E+10
	Full-Sibs						
1	PAT_1_C.fsa	727		XY	23/26		2.07E+13
2	??1.fsa	718		XY	23/26		2.07E+13
3	??3.fsa	720		XY	14/26		7.24E+04
4	PAT_3_F.fsa	685		XY	10/26		2.24E+02
5	PAT_7_F.fsa	697		XY	11/26		1.31E+02
6	PAT_5_C.fsa	739		XX	10/26		E+01
7	??.fsa	724		XY	9/26		E+01
8	PAT_5_F.fsa	740		XY	10/26		E+01
9	PAT_6_C.fsa	710		XY	10/26		E+01
10	PAT_3_C.fsa	684		XY	9/26		2.86E+01
	Half-Sibs						
1	??2.fsa	719		XY	12/26		5.73E+06
2	??.fsa	717		XY	14/26		5.06E+05
3	PAT_1_F.fsa	728		XY	14/26		5.06E+05
4	PAT_1_M.fsa	729		XX	12/26		1.85E+05
5	??.fsa	721		XX	12/26		1.85E+05
6	PAT_2_M.fsa	683		XX	10/26		2.22E+04
7	PAT_5_F.fsa	691		XY	11/26		1.21E+04
8	??.fsa	722		XX	11/26		1.04E+04
9	PAT_7_C.fsa	696		XY	12/26		9.08E+03
10	PAT_5_C.fsa	690		XX	11/26		5.33E+03

Saving Genotypes from Two or More Multiplexes to the Database

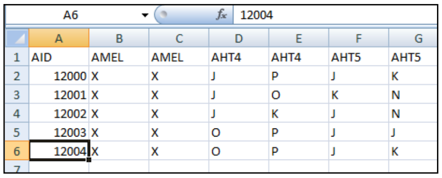
It is often necessary to run two or more multiplexes due to marker overlap or incompatible PCR conditions for different primer sets. Save the files with combined genotypes in a .txt file and import into the Database using *Save to Database* dropdown menu. Markers and allele calls can be tab delimited or a single column can contain both allele 1 and allele 2.

Select Load from TXT and navigate to the saved .txt file to add individuals with these extended genotypes to the database.

This process has been automated in GeneMarker 1.90. Please see Chapter 8 *Merge Project Tool*

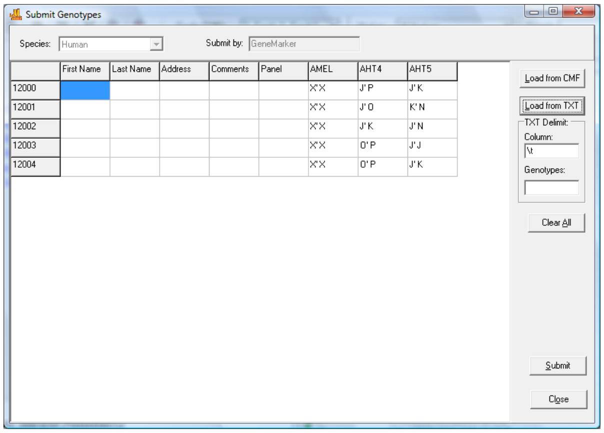


	A	B	C	D	E	F	G
1	AID	AMEL	AHT4	AHT5	ASB17	ASB2	ASB23
2	12772	XX	JP	JK	IO	NN	KS
3	12773	XX	JO	KN	RR	NN	KL
4	12774	XY	JK	JN	RR	MN	KU
5	12775	XX	OP	JJ	LR	KO	IL
6	12776	XX	OP	JK	IM	KO	KL
7							



A6 | 12004

	A	B	C	D	E	F	G
1	AID	AMEL	AMEL	AHT4	AHT4	AHT5	AHT5
2	12000	X	X	J	P	J	K
3	12001	X	X	J	O	K	N
4	12002	X	X	J	K	J	N
5	12003	X	X	O	P	J	J
6	12004	X	X	O	P	J	K
7							



Species: Human

Submit by: GeneMarker

Column: \t

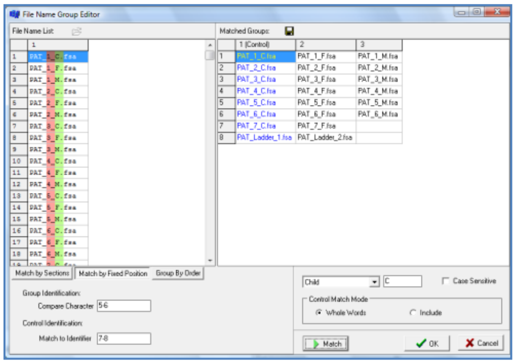
Genotypes:

	First Name	Last Name	Address	Comments	Panel	AMEL	AHT4	AHT5
12000						X'X	J'P	J'K
12001						X'X	J'O	K'N
12002						X'X	J'K	J'N
12003						X'X	O'P	J'J
12004						X'X	O'P	J'K

Parentage Verification for Pure Bred Animals

Automated Pedigree Trio Diagrams and Analysis using Family Group Tool

When a naming convention is followed the Family Group Tool enables matching of files into family groups. If naming convention was not used for these files use *File →New Pedigree* to draw the pedigree


File Name Group Editor

File Name List

	1
1	PAT_1_C.fsa
2	PAT_1_F.fsa
3	PAT_1_M.fsa
4	PAT_2_C.fsa
5	PAT_2_F.fsa
6	PAT_2_M.fsa
7	PAT_3_C.fsa
8	PAT_3_F.fsa
9	PAT_3_M.fsa
10	PAT_4_C.fsa
11	PAT_4_F.fsa
12	PAT_4_M.fsa
13	PAT_5_C.fsa
14	PAT_5_F.fsa
15	PAT_5_M.fsa
16	PAT_6_C.fsa
17	PAT_6_F.fsa
18	PAT_6_M.fsa

Matched Groups:

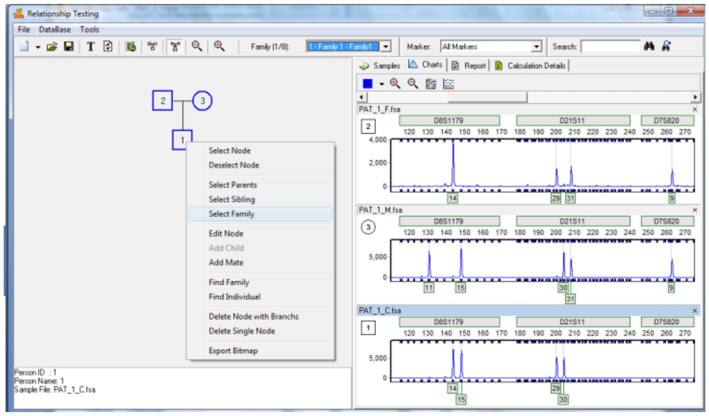
	1 (Control)	2	3
1	PAT_1_C.fsa	PAT_1_F.fsa	PAT_1_M.fsa
2	PAT_2_C.fsa	PAT_2_F.fsa	PAT_2_M.fsa
3	PAT_3_C.fsa	PAT_3_F.fsa	PAT_3_M.fsa
4	PAT_4_C.fsa	PAT_4_F.fsa	PAT_4_M.fsa
5	PAT_5_C.fsa	PAT_5_F.fsa	PAT_5_M.fsa
6	PAT_6_C.fsa	PAT_6_F.fsa	PAT_6_M.fsa
7	PAT_7_C.fsa	PAT_7_F.fsa	
8	PAT_Ladder_1.fsa	PAT_Ladder_2.fsa	

Group Identification:
Compare Character: 5-6
Control Identification:
Match to Identifier: 7-8
Child: C
☐ Case Sensitive
Control Match Mode: ● Whole Words ○ Include

Match by Sections
Match by Fixed Position
Group By Order
Match
OK
Cancel

Procedure:

1. Select *Applications → Relationship Testing* from the menu bar of the *Main Analysis* window
2. Match by Sections, Positions or Group Order and then Match Whole Words → OK
3. The Pedigree for the families is drawn and displayed at the left of the Sample List.


Relationship Testing

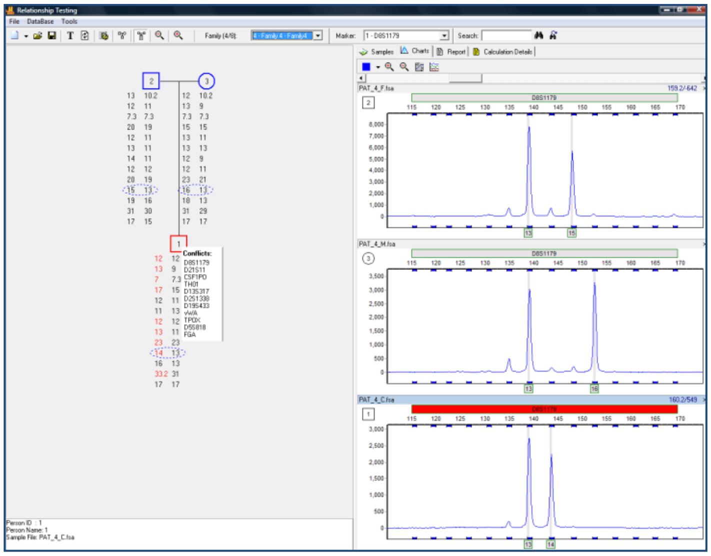
Family (1/8): 1 - Family 1 - Family1
Marker: All Markers

Person ID : 1
Person Name: 1
Sample File: PAT_1_C.fsa

File
DataBase
Tools
Search:
Samples
Charts
Report
Calculation Details
Select Node
Deselect Node
Select Parents
Select Sibling
Select Family
Edit Node
Add Child
Add Mate
Find Family
Find Individual
Delete Node with Branchs
Delete Single Node
Export Bitmap
PAT_1_F.fsa
PAT_1_M.fsa
PAT_1_C.fsa
D8S1179
D21S11
D7S820
2
3
1

4. Right click on a node for edit or analysis options.
5. *Select Family* displays all electropherograms for the pedigree tree at the right.
6. *Select Node* or parents, siblings displays electropherograms
7. *Edit Node* allows editing of file or electropherogram information – Be sure to use the refresh key after any changes
8. *Add Mate or child* to expand the pedigree

Allele conflicts are listed and the node is highlighted in red. Clicking on the marker in the list links to the section of the electropherogram where the conflict can be visualized.


Relationship Testing

Family (4/8): 4 - Family 4 - Family4
Marker: 1 - D8S1179

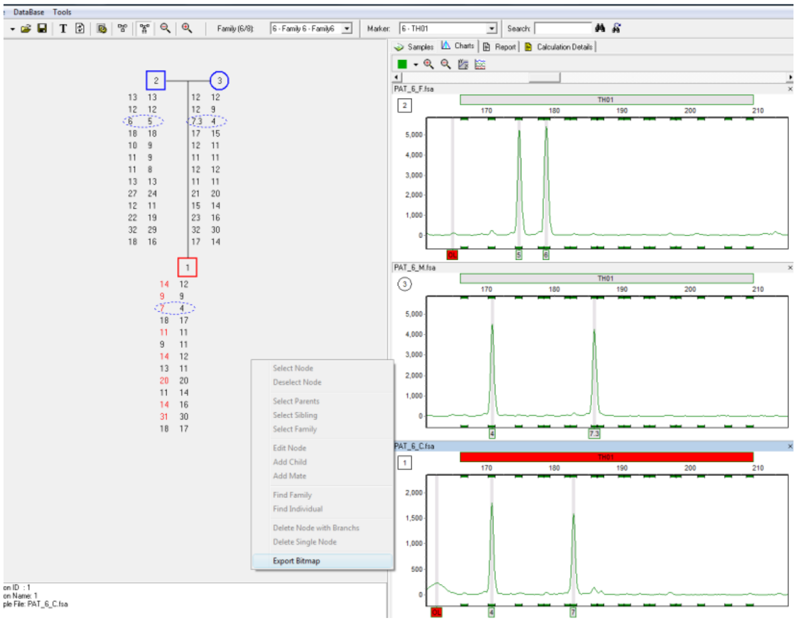
Conflicts:
D8S1179
D21S11
TH01
CSF1PO
D13S317
D2S1338
D19S433
vWA
TPOX
D5S818
FGA

Person ID : 1
Person Name: 1
Sample File: PAT_4_C.fsa

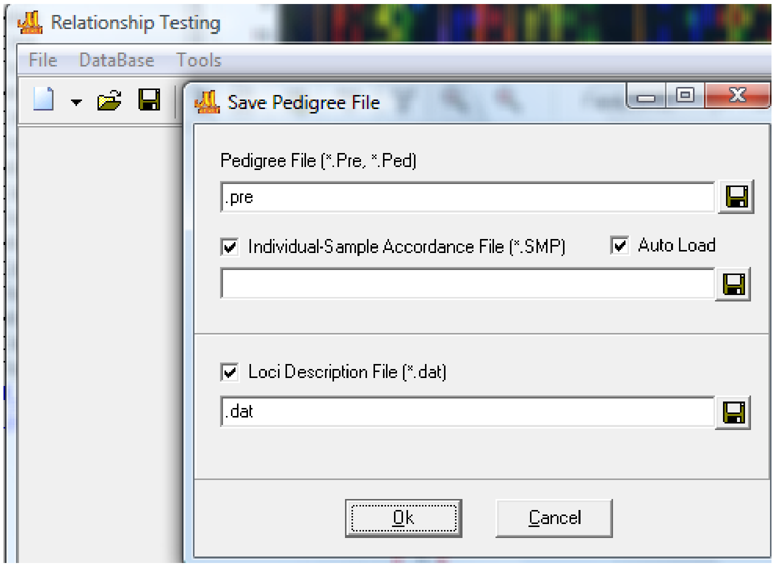
File
DataBase
Tools
Search:
Samples
Charts
Report
Calculation Details
PAT_4_F.fsa
PAT_4_M.fsa
PAT_4_C.fsa
D8S1179
2
3
1

Save Report

Export Bitmap to save the diagram


DataBase
Tools
Family (6/8)
6 - Family 6 - Family6
Marker:
6 - TH01
Search:
Samples
Charts
Report
Calculation Details
PAT_6_F.fsa
TH01
PAT_6_M.fsa
PAT_6_C.fsa
Select Node
Deselect Node
Select Parents
Select Sibling
Select Family
Edit Node
Add Child
Add Mate
Find Family
Find Individual
Delete Node with Branchs
Delete Single Node
Export Bitmap

Save pedigree drawings with the save icon. Pedigrees may be re-opened and edited as more information becomes available.


Relationship Testing
File
DataBase
Tools
Save Pedigree File
Ok
Cancel

Pedigree File (*.Pre, *.Ped)

.pre

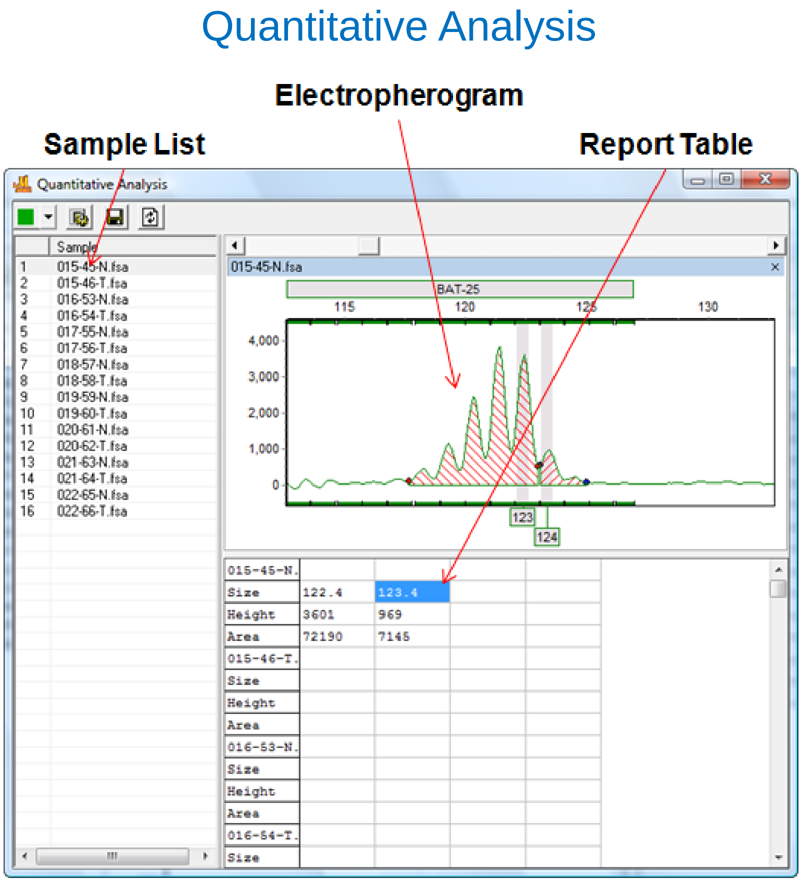
☑ Individual-Sample Accordance File (*.SMP) ☑ Auto Load

☑ Loci Description File (*.dat)

.dat

Quantitative Analysis

The *Quantitative Analysis* feature determines areas under the curve of complex peaks. Two different analysis methods are included: the *Curtain Method* and the *Deconvolution Method*. The *Curtain Method* allows the user to define the start and end range for area calculation under a peak curve. The *Deconvolution Method* compares a selected sample's peak areas to the peak areas of the other samples at the same position.



Quantitative Analysis

Electropherogram

Sample List

Report Table

	Sample
1	015-45-N.fsa
2	015-46-T.fsa
3	016-53-N.fsa
4	016-54-T.fsa
5	017-55-N.fsa
6	017-56-T.fsa
7	018-57-N.fsa
8	018-58-T.fsa
9	019-59-N.fsa
10	019-60-T.fsa
11	020-61-N.fsa
12	020-62-T.fsa
13	021-63-N.fsa
14	021-64-T.fsa
15	022-65-N.fsa
16	022-66-T.fsa

015-45-N.				
Size	122.4	123.4		
Height	3601	969		
Area	72190	7145		
015-46-T.				
Size				
Height				
Area				
016-53-N.				
Size				
Height				
Area				
016-54-T.				
Size				

Sample List

The *Sample List* displays all current samples in the project. Single left-click a sample name or use the **Up/Down Arrow** keys to scroll through the list.

Electropherogram

The *Electropherogram* frame displays the currently selected sample's trace with either *Curtain* shaded areas or *Deconvolution* dashed trace overlay. Allele editing options are similar to the *Main Analysis* window *Electropherogram* frame. **See Chapter 3 Main Analysis Overview**.

Report Table

The *Report Table* shows the size, height and area calculations for the peaks under analysis. Adjusting the start and end range points with the *Curtain Method* will alter the area value for the allele.

Procedure

1. Import raw data files and filter with Run Wizard. **See Chapter 2 General Procedure.**

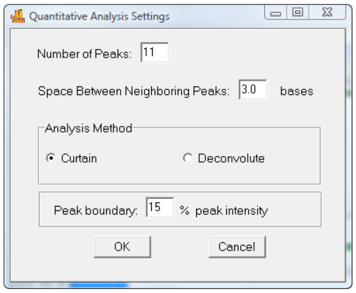
NOTE: A Panel does not need to be applied to the dataset to use the Quantitative Analysis module.

2. Select *Applications → Quantitative Analysis*
3. The *Quantitative Analysis* window appears
4. Click the **Select Peak Number and Space** icon
5. The *Quantitative Analysis Settings* box will appear
6. Choose either *Curtain* or *Deconvolute* analysis method

7. Click **OK**
8. The analysis method chosen is applied
9. Edit the results and click **Refresh** to update the *Report Table*
10. Click **Save Report** to export the *Report Table* as a tab-delimited Text (.txt) file

Icons and Functions

Quantitative Analysis Settings
Activates the *Quantitative Analysis Settings* dialog box.



Number of Peaks: 11

Space Between Neighboring Peaks: 3.0 bases

● Curtain ○ Deconvolute

Peak boundary: 15 % peak intensity

Number of Peaks
Allows the user to select the number of peaks that are expected in the region.

Space Between Neighboring Peaks
Provides the option to specify the number of base pairs that separate the expected peaks.

Analysis Method
The user can choose between the *Curtain* and *Deconvolute* methods. See *What to Expect* section below.

Peak Boundary (% Peak Intensity)
Sets the start and end range points at the RFU position on the trace that equals the percentage RFU value of the highest peak.

Save Report
Saves the *Quantitative Analysis Report Table* information as a Text (.txt) file.

Refresh
Calculates the values of the added peaks and updates the *Quantitative Analysis Report Table*.

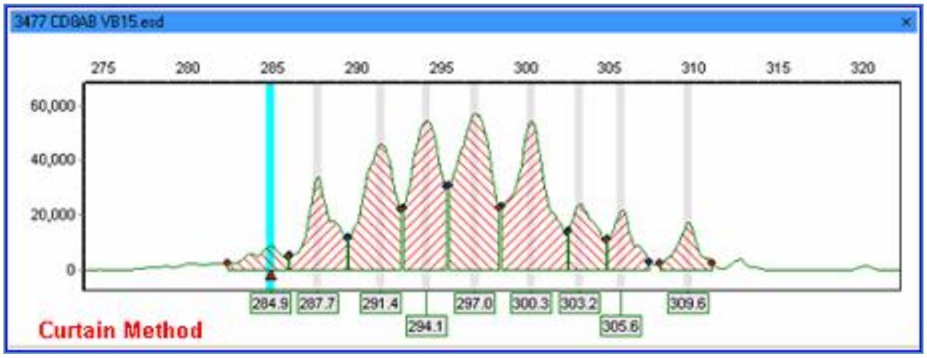
What to Expect

Curtain Method

The Curtain method is best for very complex traces, as it is difficult to calculate the area for a primary peak when secondary peaks exist. Using this method, primary peaks will be calculated, while secondary peaks will be removed from the calculation and will be left in an unassigned area between the peak boundaries. This method integrates to calculate the area under each individual peak in the region. The analysis shows gaps between the peaks in the region, so the region between the peaks is not assigned to either peak for the area calculation. To set the area between the peaks, input the desired value in the **Peak Boundary: 15 % peak intensity** field. A point along the curve must be at least x% of the primary peak's intensity in order to be assigned as part of the primary peak, and is used to calculate the area under that peak.

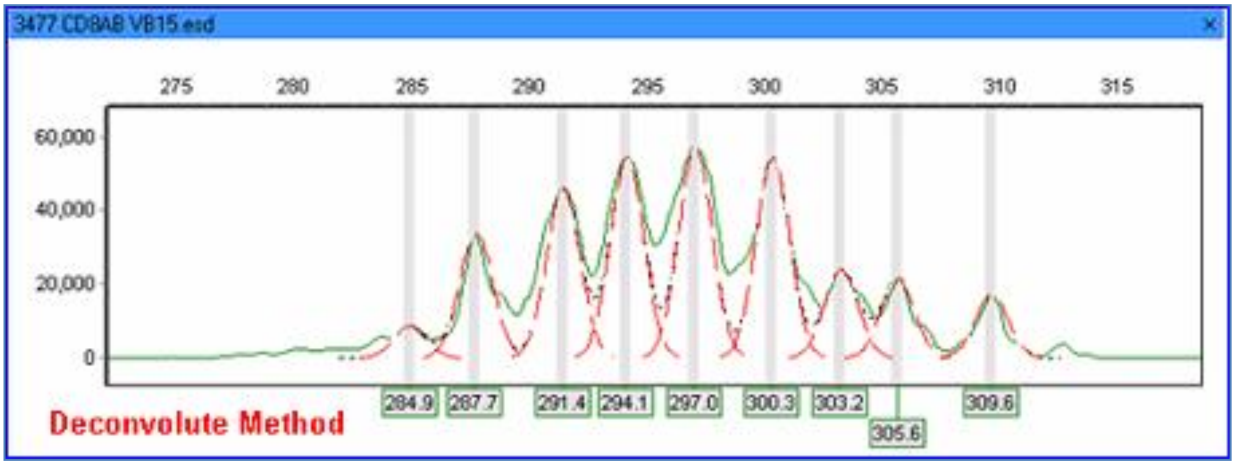
Editing Peak Boundaries

1. Hold down the **Ctrl** key and use the left-mouse button to move the peak boundary.
2. The peak boundary will be marked by a red diamond.
3. After setting the new boundary, release the key and mouse button.
4. The new area will be automatically calculated.



Deconvolute Method

The Deconvolute method is best for very good data with only a few, small secondary peaks. This method allows users to view the **Individual Peaks** as well as the **Complex Peaks**. The area calculation performed is only for the Individual peaks, but the Complex peaks can be shown to give the user a general idea of the Complex Peak shape and distribution. The Deconvolute method calculates the area under each peak, including some of the area in between the peaks. This method calculates a value by assigning the regions between the peaks to one peak or the other.



Single Nucleotide Polymorphism (SNP) Analysis

Single nucleotide polymorphisms (SNPs) occur every 100 to 300 bases along the human genome and make up to 90% of human genetic variation. Functional SNPs—classified as non-synonymous SNPs (nsSNPs) that occur in the coding region of a gene or as regulatory SNPs (rSNPs) that occur in the promoter region of a gene—are often associated with altered protein function or gene expression. Intronic or intergenic SNPs may not alter gene or protein function, but can be used to address questions in evolutionary biology or in association studies with complex diseases, drug response, environmental insults quantitative trait loci (QTL) or genotyping plants and animals.

Various techniques have been developed to interrogate SNPs, including SNPlex™, SNaPshot™, SNuPE™, SNPWave®, SNP chips and DNA sequence analysis.

SNaPshot & SNuPE

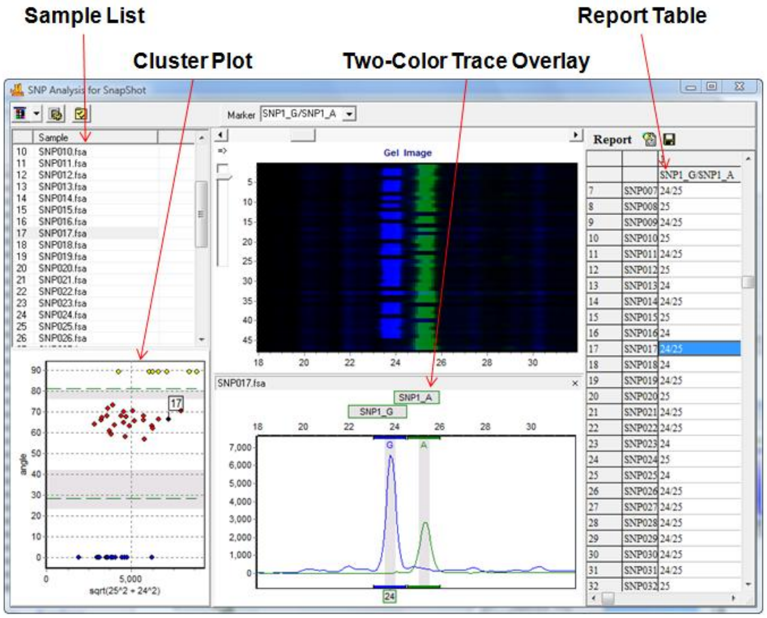
One method to determine SNP genotypes is single base extension or SBE. An unlabeled primer with its 3’ end directly flanking the SNP is extended one nucleotide by Taq polymerase and fluorescently-labeled ddNTPs complementary to the polymorphic base are added. The resulting fragment is one nucleotide longer, but the observed fragment size on a gel will be greater than expected due to the influence of the fluorescent dye on the electrophoretic mobility of these small fragments. SNPs can be identified by the one- or two-color peaks associated with the incorporated labeled ddNTP and the length of the primer. Primer extension method has the advantage of accurate genotyping using a low number of unlabeled user-defined primers.

The SBE technique can quickly interrogate a small number of SNPs. Two commonly used kits for SBE technique are the MegaBACE™ SNuPE Genotyping Kit (Amersham Bioscience) and the SNaPshot Genotyping System (Applied Biosystems). To fully utilize the investigative potential of SBE, a robust genotyping and data analysis system should be employed. GeneMarker genotyping software is designed for fast, accurate and efficient analysis and reporting of primer extension data.

Overview

The SNP analysis report window displays a synthetic gel image, list of samples, cluster plot to analyze peak information and assign SNP genotypes, and sample electropherogram. The information contained in the report is interlinked. Double-clicking on the cell in the report highlights the cell, corresponding sample ID in the sample list, data point in the cluster plot and locus in the electropherogram.

SNaPshot Analysis



	Sample
10	SNP010.fsa
11	SNP011.fsa
12	SNP012.fsa
13	SNP013.fsa
14	SNP014.fsa
15	SNP015.fsa
16	SNP016.fsa
17	SNP017.fsa
18	SNP018.fsa
19	SNP019.fsa
20	SNP020.fsa
21	SNP021.fsa
22	SNP022.fsa
23	SNP023.fsa
24	SNP024.fsa
25	SNP025.fsa
26	SNP026.fsa

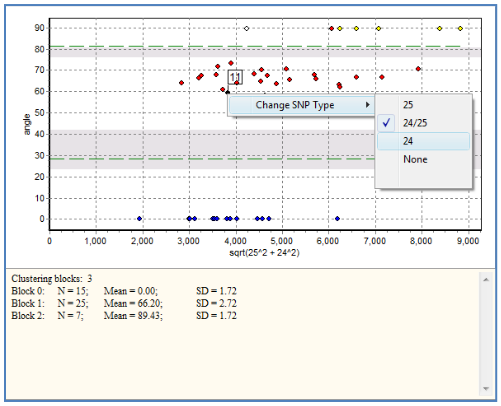
		SNP1_G/SNP1_A
7	SNP007	24/25
8	SNP008	25
9	SNP009	24/25
10	SNP010	25
11	SNP011	24/25
12	SNP012	25
13	SNP013	24
14	SNP014	24/25
15	SNP015	25
16	SNP016	24
17	SNP017	24/25
18	SNP018	24
19	SNP019	24/25
20	SNP020	25
21	SNP021	24/25
22	SNP022	24/25
23	SNP023	24
24	SNP024	25
25	SNP025	24
26	SNP026	24/25
27	SNP027	24/25
28	SNP028	24/25
29	SNP029	24/25
30	SNP030	24/25
31	SNP031	24/25
32	SNP032	25

Sample List

The *Sample List* displays all current samples in the project. Single left-click a sample name or use the **Up/Down Arrow** keys to scroll through the list.

Cluster Plot

The *Cluster Plot* displays, on a graph of peak angle vs. Marker position, points representing each sample. Choose to show the *Cluster Plot* in *Cartesian* or *Polar* format in the *Display Settings* box. Select a Marker from the *Marker* drop-down menu in the toolbar to view its *Cluster Plot*. Click on points in the graph to see individual sample traces in the *Two-Color Trace Overlay* frame. Plot points are separated into groups by green dashed lines. The green group separation lines are derived from a statistical cluster analysis of the specific dataset and therefore vary for each Marker. Points between the green separation lines represent samples where both alleles are present in the Marker. Points above and below the green separation lines contain only one peak or allele. Statistical information about the *Cluster Plot* can be displayed by selecting *Show Clustering Information* in the *Display Settings* box.



```
Clustering blocks:  3
Block 0:    N = 15;     Mean = 0.00;        SD = 1.72
Block 1:    N = 25;     Mean = 66.20;       SD = 2.72
Block 2:    N = 7;      Mean = 89.43;       SD = 1.72
```

Right-click at a point in the *Cluster Plot* to modify the sample's allele call at the selected Marker. A fly-out menu will appear with options to change the SNP type. When the SNP type is changed in the *Cluster Plot*, the *Report Table* is automatically updated.

Two-Color Trace Overlay

The *Two-Color Trace Overlay* displays the electropherogram traces for two associated dye colors based on the Marker naming convention used when the Panel was created. See *SNaPshot Panel Creation* section below. Since SNaPshot uses single base extension technique, expect the associated alleles to be separated by approximately one base pair with some deviation with regard to the different dye sizes and weights. The allele editing options in the *Two-Color Trace Overlay* frame are similar to the *Main Analysis* window *Electropherogram* options. **See Chapter 3 Main Analysis Overview**.

Report Table

The Report Table lists the sample filenames in rows in the first column on the left. The associated Markers appear as column headers. Alleles in two dye colors are combined into one Marker by the Marker's naming convention. See the *SNaPshot Panel Creation* section below. Be default, the detected alleles are reported in the table. Other value display options include *Peak Ratio* and *Probability*. See the *SNP Analysis Reporting* section below.

Procedure

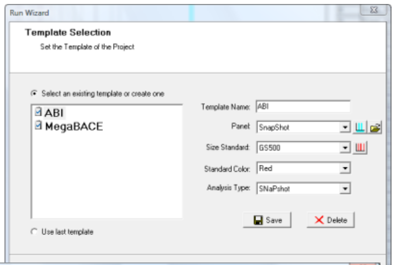
SNP analysis in GeneMarker requires the data to be sized and a Panel applied prior to launching the SNPlex/SNaPshot module. SNaPshot specifically requires the association of dye color to expected nucleotide which can be accomplished in the *Panel Editor*. Below is the procedure for filtering with Run Wizard and creating a SNaPshot Panel.

SNaPshot Run Wizard Settings

For explanation of specific functions in Run Wizard, **see Chapter 2 General Procedure**.

Run Wizard Template Selection

Panel: NONE OR chose custom Panel
Size Standard: User-defined
Analysis Type: SNaPshot
NOTE: A Panel is required to analyze SNaPshot data. Choose NONE in the Panel field only if a Panel has not yet been created.



Run Wizard

Template Selection

Set the Template of the Project

● Select an existing template or create one

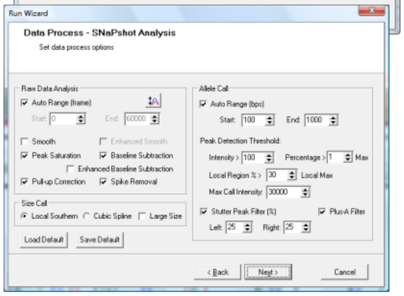
ABI
MegaBACE

Template Name: ABI
Panel: SnapShot
Size Standard: GS500
Standard Color: Red
Analysis Type: SNaPshot

○ Use last template

Run Wizard Data Process

Peak Detection Intensity Threshold: 100
Global Percentage: 1% Max
Local Percentage: 30%
Max Call Intensity: 30,000
Stutter Peak Filter: Left: 25% Right: 25%
Plus A Filter: Selected



Run Wizard

Data Process - SNaPshot Analysis

Set data process options

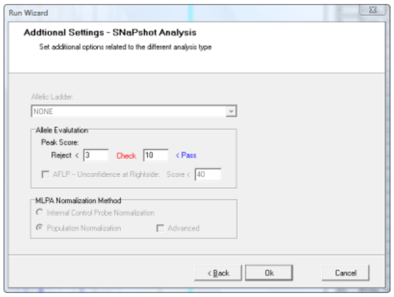
Raw Data Analysis
☑ Auto Range (frame)
Start: 0 End: 60000
☐ Smooth ☐ Enhanced Smooth
☑ Peak Saturation ☑ Baseline Subtraction
☐ Enhanced Baseline Subtraction
☑ Pull-up Correction ☑ Spike Removal

Size Call
● Local Southern ○ Cubic Spline ☐ Large Size

Allele Call
☑ Auto Range (bps)
Start: 100 End: 1000
Peak Detection Threshold:
Intensity > 100 Percentage > 1 Max
Local Region % > 30 Local Max
Max Call Intensity: 30000
☑ Stutter Peak Filter (%) ☑ Plus-A Filter
Left: 25 Right: 25

Run Wizard Additional Settings

Allele Evaluation Peak Score: Reject < 3 Check 10 < Pass
NOTE: If the Reject value is set too high, a false negative call could result. If the Reject value is set too low, then a false positive call may occur.



Run Wizard

Addtional Settings - SNaPshot Analysis

Set additional options related to the different analysis type

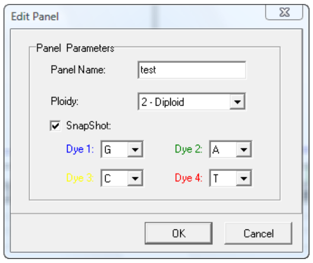
Allelic Ladder: NONE

Allele Evalutation
Peak Score:
Reject < 3 Check 10 < Pass
☐ AFLP -- Unconfidence at Rightside: Score < 40

MLPA Normalization Method
○ Internal Control Probe Normalization
● Population Normalization ☐ Advanced

SNaPshot Panel Creation

After the data has been sized and filters have been applied, go to *Tools* → *Panel Editor* to create a Panel for the dataset. For explanation of specific functions in *Panel Editor*, **see Chapter 5 Panel Editor**.


Edit Panel
Panel Parameters
OK
Cancel

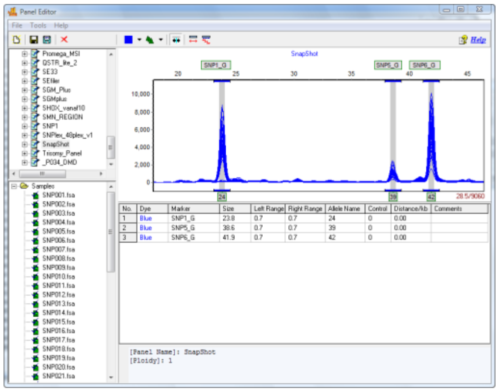
Panel Name: test

Ploidy: 2 - Diploid

☑ SnapShot:

Dye 1: G
Dye 2: A
Dye 3: C
Dye 4: T

1. In *Panel Editor*, select *File* → *Create New Panel* <u>OR</u> click the **Create New Panel** icon
2. The *Create New Panel* box appears
3. Enter a name for the Panel and be sure **SNaPshot** is selected in the *Type* field
4. Select *Automatically Create* and *Use All Samples*
5. Click **OK**
6. *Panel Editor* will place Markers in each dye color where peaks are detected
7. Right-click the panel name in the *Panel List*
8. Select *Edit*
9. The *Edit Panel* box will appear
10. Set the *Ploidy* to **2-Diploid**
11. Select **SNaPshot** and associate dye colors with nucleotides
12. Click **OK**


Panel Editor
File
Tools
Help
Help
Promega_MSI
QSTR_lite_2
SE33
SEfiler
SGM_Plus
SGMplus
SHOX_vanaf10
SMN_REGION
SNP1
SNPlex_48plex_v1
SnapShot
Trisomy_Panel
_P034_DMD
Samples
SNP001.fsa
SNP002.fsa
SNP003.fsa
SNP004.fsa
SNP005.fsa
SNP006.fsa
SNP007.fsa
SNP008.fsa
SNP009.fsa
SNP010.fsa
SNP011.fsa
SNP012.fsa
SNP013.fsa
SNP014.fsa
SNP015.fsa
SNP016.fsa
SNP017.fsa
SNP018.fsa
SNP019.fsa
SNP020.fsa
SNP021.fsa
SnapShot
SNP1_G
SNP5_G
SNP6_G
20
25
30
35
40
45
10,000
8,000
6,000
4,000
2,000
0
24
39
42
28.5/9060

No.	Dye	Marker	Size	Left Range	Right Range	Allele Name	Control	Distance/kb	Comments
1	Blue	SNP1_G	23.8	0.7	0.7	24	0	0.00	
2	Blue	SNP5_G	38.6	0.7	0.7	39	0	0.00	
3	Blue	SNP6_G	41.9	0.7	0.7	42	0	0.00	

```
[Panel Name]: SnapShot
[Ploidy]: 1
```

13. In the *Overlay Trace* frame, rename the Markers so that GeneMarker can associate SNPs in two different dye colors. For example, if primer-1 was designed to detect SNPs A and G of BRCA1, an acceptable naming protocol would be to name one Marker peak of the pair as SNP1_A or BRCA1a_A in the green dye color and the other Marker peak as SNP1_G or BRCA1a_G in the blue dye color.

NOTE: The naming schema is case and punctuation sensitive.

14. Select *File* → *Save Changes* (**Hot Key** = **CTRL+S**) OR click **Save Changes** icon
15. Exit the *Panel Editor*
16. Click the **Run Process** icon in the *Main Analysis* window
17. Select the newly created Panel from the Run Wizard *Template Selection Panel* drop-down menu
18. Proceed through Run Wizard and click **OK** in the *Data Process* box
19. The Panel has been applied
20. Select *Applications* → *SNPlex/SNaPshot*
21. The *SNaPshot Analysis* window appears

Icons and Functions

Show Dye

Left-click to show individual colors in the *Two-Color Trace Overlay* or use drop-down menu to select *Show All* or *Hide All* dye colors.

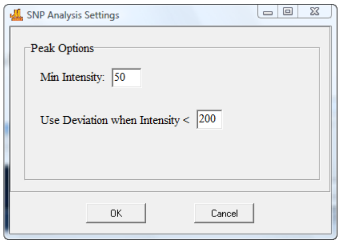
SNP Analysis Settings

Launches the *SNP Analysis Settings* box.

Peak Options

<u>*Min Intensity*</u>: Peaks with RFU heights below this value will not be called.

<u>*Use Deviation when Intensity*</u>: Peaks with RFU heights below this value will be called with higher sensitivity using a deviation method to remove the influence of the baseline.


SNP Analysis Settings
Peak Options
OK
Cancel

Min Intensity: 50

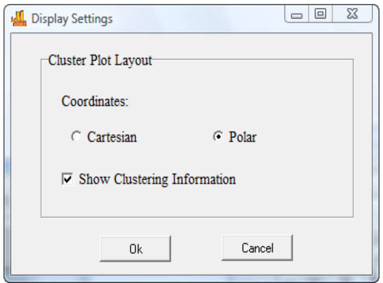
Use Deviation when Intensity < 200

Layout Settings

Launches the *Display Settings* box.

Cluster Plot Layout

<u>*Coordinates Cartesian/Polar*</u>: Select the format in which the *Cluster Plot* will be displayed. *Polar* is the default and displays the angle of the


Display Settings
Cluster Plot Layout
Ok
Cancel

Coordinates:

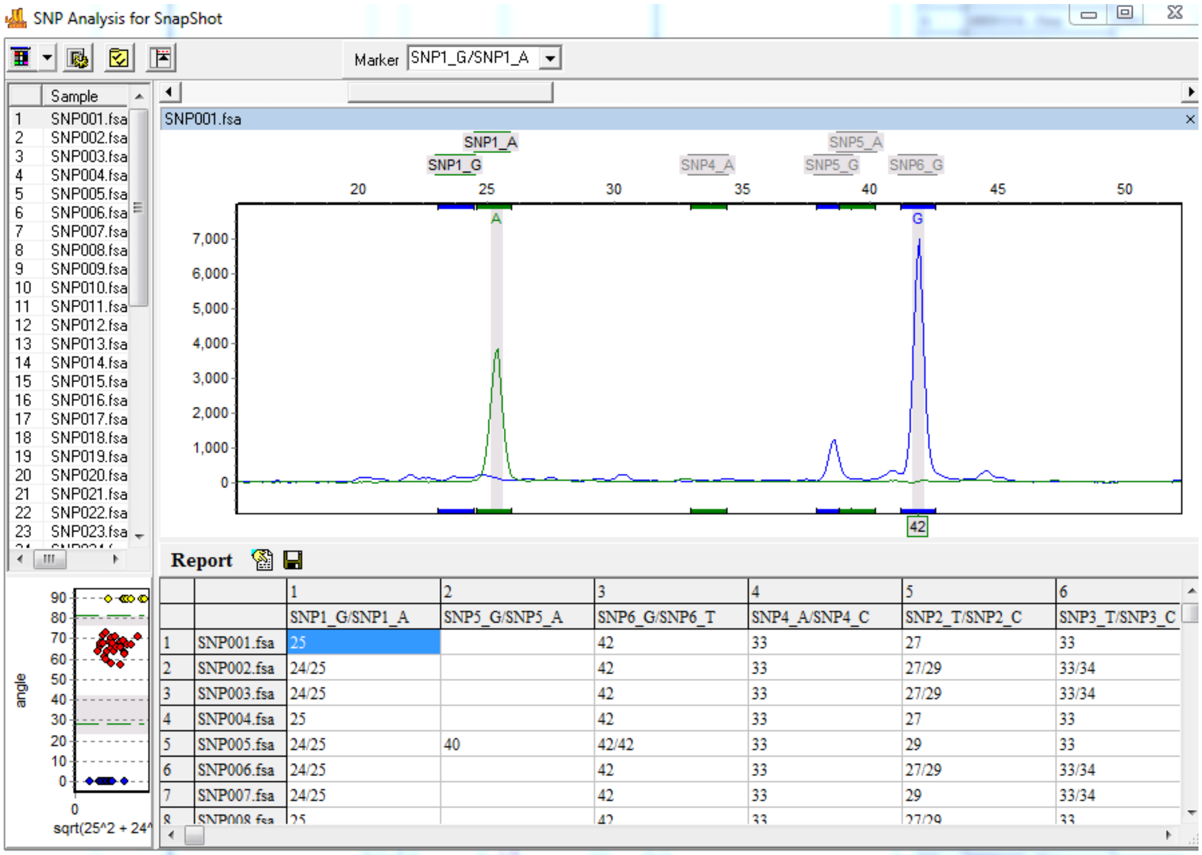
○ Cartesian ● Polar

☑ Show Clustering Information

vector connecting the peak intensities of the two alleles versus the square root of the sum of the square of the longer fragments and the square of the shorter fragment. *Cartesian* displays the plot points on an Allele 1 versus Allele 2 graph.

Show Clustering Information: When selected, a statistical table will appear below the *Cluster Plot* with information for each group of plot points. Statistical information reported includes number of samples in the group's population, mean of the group, and standard deviation (SD) of the group.

Hide Synthetic Gel Image – and display the report table under the electropherogram


SNP Analysis for SnapShot
Marker
SNP1_G/SNP1_A
Sample
1 SNP001.fsa
2 SNP002.fsa
3 SNP003.fsa
4 SNP004.fsa
5 SNP005.fsa
6 SNP006.fsa
7 SNP007.fsa
8 SNP008.fsa
9 SNP009.fsa
10 SNP010.fsa
11 SNP011.fsa
12 SNP012.fsa
13 SNP013.fsa
14 SNP014.fsa
15 SNP015.fsa
16 SNP016.fsa
17 SNP017.fsa
18 SNP018.fsa
19 SNP019.fsa
20 SNP020.fsa
21 SNP021.fsa
22 SNP022.fsa
23 SNP023.fsa
SNP001.fsa
SNP1_A
SNP1_G
SNP4_A
SNP5_A
SNP5_G
SNP6_G
20
25
30
35
40
45
50
A
G
7,000
6,000
5,000
4,000
3,000
2,000
1,000
0
42
angle
90
80
70
60
50
40
30
20
10
0
0
sqrt(25^2 + 24^
Report

		1	2	3	4	5	6
		SNP1_G/SNP1_A	SNP5_G/SNP5_A	SNP6_G/SNP6_T	SNP4_A/SNP4_C	SNP2_T/SNP2_C	SNP3_T/SNP3_C
1	SNP001.fsa	25		42	33	27	33
2	SNP002.fsa	24/25		42	33	27/29	33/34
3	SNP003.fsa	24/25		42	33	27/29	33/34
4	SNP004.fsa	25		42	33	27	33
5	SNP005.fsa	24/25	40	42/42	33	29	33
6	SNP006.fsa	24/25		42	33	27/29	33/34
7	SNP007.fsa	24/25		42	33	29	33/34
8	SNP008.fsa	25		42	33	27/29	33

The report table is located beneath the electropherogram when the gel image is de-selected. Only peaks that fall within the bins of the panel are called in the SNP Analysis screen (providing 'Abide by Panel' functionality).

What to Expect

SNaPshot and SNuPE implement single base extension technique by dye-labeling primers with different colors for the different expected SNP alleles. So, in addition to the complimentary fragment appearing one basepair larger, the different dye molecules will affect the mobility of the fragments differently. This slight difference must be taken into account when creating Bins in the *Panel Editor*. It is recommended to expand the Bin range slightly (~0.7) on the *Left* and *Right* to accommodate variable mobility.

In the final *SNaPshot Analysis* window, plot points in the *Cluster Plot* that approach the green group separation lines should be examined individually for accuracy. After each Marker has been verified, export the Report Table – see the *SNP Analysis Reporting* section below.

SNPlex

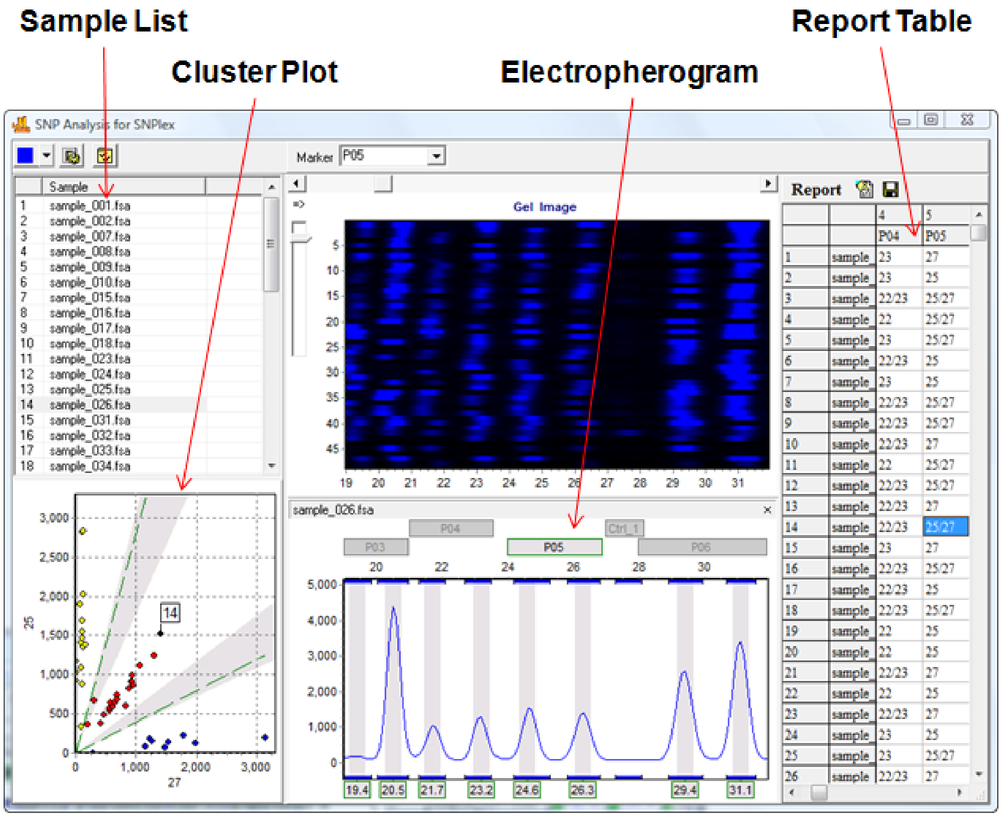
To fully utilize the diagnostic potential of SNPs, a robust high-throughput genotyping and data analysis system should be employed. SNPlex Genotyping System (Applied Biosystems) can interrogate 48 SNPs simultaneously and has been used to investigate SNPs in 92 cancer-related genes in breast cancer and to genotype plants. GeneMarker genotyping software is designed for fast, accurate and efficient analysis of SNPlex data.

Overview

The SNPlex Analysis window displays a synthetic gel image, list of samples, cluster plot to analyze peak information and assign SNP genotypes, sample electropherogram and SNP genotyping report. The information contained in the report is interlinked. Double-clicking on the cell highlights the cell, corresponding sample ID in the sample list, data point in the cluster plot and locus in the electropherogram.

The *Sample List*, *Cluster Plot*, and *Report Table* in SNPlex analysis are all similar to SNaPshot analysis. See SNaPshot/SNuPe section above for explanation of functionality. The *Electropherogram* frame in SNPlex analysis differs from SNaPshot analysis. SNPlex technique uses primer length variation to identify SNP pairs. SNP pairs in SNPlex analysis are typically separated by approximately two bases, therefore an entire 48-plex SNP analysis can be accomplished with just two dye colors.

SNPlex Analysis


Sample List
Cluster Plot
Electropherogram
Report Table
SNP Analysis for SNPlex
Marker
P05
Gel Image
5
10
15
20
25
30
35
40
45
19
20
21
22
23
24
25
26
27
28
29
30
31
sample_026.fsa
P03
P04
P05
Ctrl_1
P06
5,000
4,000
3,000
2,000
1,000
0
19.4
20.5
21.7
23.2
24.6
26.3
29.4
31.1
3,000
2,500
2,000
1,500
1,000
500
0
25
1,000
2,000
3,000
27
14
Report

	Sample
1	sample_001.fsa
2	sample_002.fsa
3	sample_007.fsa
4	sample_008.fsa
5	sample_009.fsa
6	sample_010.fsa
7	sample_015.fsa
8	sample_016.fsa
9	sample_017.fsa
10	sample_018.fsa
11	sample_023.fsa
12	sample_024.fsa
13	sample_025.fsa
14	sample_026.fsa
15	sample_031.fsa
16	sample_032.fsa
17	sample_033.fsa
18	sample_034.fsa

		4	5
		P04	P05
1	sample_	23	27
2	sample_	23	25
3	sample_	22/23	25/27
4	sample_	22	25/27
5	sample_	23	25/27
6	sample_	22/23	25
7	sample_	23	25
8	sample_	22/23	25/27
9	sample_	22/23	25/27
10	sample_	22/23	27
11	sample_	22	25/27
12	sample_	22/23	25/27
13	sample_	22/23	27
14	sample_	22/23	25/27
15	sample_	23	27
16	sample_	22/23	25/27
17	sample_	22/23	25
18	sample_	22/23	25/27
19	sample_	22	25
20	sample_	22	25
21	sample_	22/23	27
22	sample_	22	25
23	sample_	22/23	27
24	sample_	23	25
25	sample_	23	25/27
26	sample_	22/23	27

Procedure

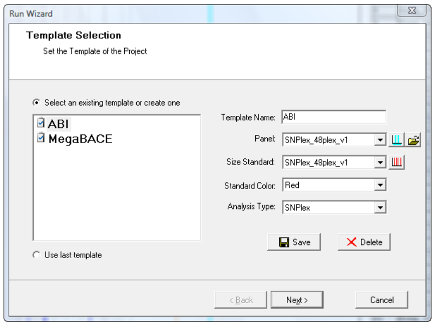
SNP analysis in GeneMarker requires the data to be sized and a Panel applied prior to launching the SNPlex/SNaPshot module. The ABI SNPlex Panel comes standard with GeneMarker and can be found in the pre-defined *Panel List*.

SNPlex Run Wizard Settings

For explanation of specific functions in Run Wizard, **see Chapter 2 General Procedure**.

Run Wizard Template Selection

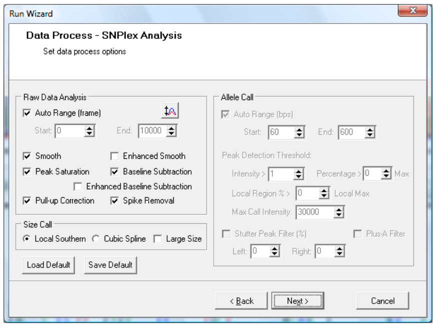
Panel: SNPlex_48Plex_v1
Size Standard: SNPlex_48Plex_v1
Analysis Type: SNPlex
NOTE: A Panel is required to analyze SNPlex data.


Run Wizard
Template Selection
Set the Template of the Project
Save
Delete
< Back
Next >
Cancel

● Select an existing template or create one
☑ ABI
☑ MegaBACE
○ Use last template
Template Name: ABI
Panel: SNPlex_48plex_v1
Size Standard: SNPlex_48plex_v1
Standard Color: Red
Analysis Type: SNPlex

Run Wizard Data Process & Additional Settings

SNPlex analysis does not require *Allele Call* or *Peak Score Threshold* settings to be adjusted; therefore, the settings are inactive in the Run Wizard.


Run Wizard
Data Process - SNPlex Analysis
Set data process options
Raw Data Analysis
Allele Call
Size Call
Load Default
Save Default
< Back
Next >
Cancel

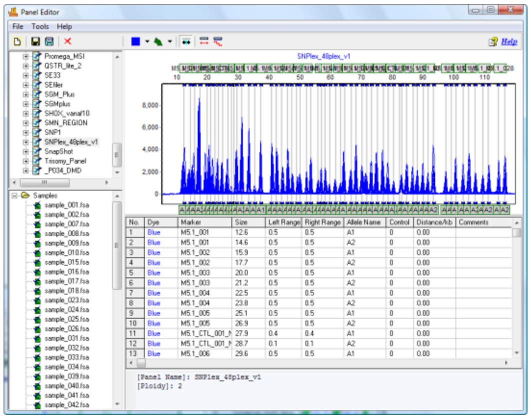
☑ Auto Range (frame)
Start: 0 End: 10000
☑ Smooth ☐ Enhanced Smooth
☑ Peak Saturation ☑ Baseline Subtraction
☐ Enhanced Baseline Subtraction
☑ Pull-up Correction ☑ Spike Removal
● Local Southern ○ Cubic Spline ☐ Large Size
☑ Auto Range (bps)
Start: 60 End: 600
Peak Detection Threshold:
Intensity > 1 Percentage > 0 Max
Local Region % > 0 Local Max
Max Call Intensity: 30000
☐ Stutter Peak Filter (%) ☐ Plus-A Filter
Left: 0 Right: 0

SNPlex Panel Adjustment

1. After the data has been sized, select *Tools → Panel Editor*
2. Select *SNPlex_48plex_v1* Panel from the *Panel List*
3. Deselect the **Check Range in Edit** icon
4. Edit Markers and Bins to align with the dataset peaks. **See Chapter 5 Panel Editor**.

NOTE: Each Marker contains two Bins – *Allele 1* and *Allele 2*.

5. Select *File → Save Changes* **(Hot Key = CTRL+S)** *OR* click **Save Changes** icon
6. Exit the *Panel Editor*
7. Select *Project → AutoRun* from the main menu bar
8. Click **OK** in the *Data Process* box
9. The adjusted Panel has been applied
10. Select *Applications → SNPlex/SNaPshot*
11. The *SNPlex Analysis* window appears


Panel Editor
File Tools Help
Help
Promega_MSI
QSTR_lite_2
SE33
SEfiler
SGM_Plus
SGMplus
SHOX_vanaf10
SMN_REGION
SNP1
SNPlex_48plex_v1
SnapShot
Trisomy_Panel
_P034_DMD
Samples
sample_001.fsa
sample_002.fsa
sample_007.fsa
sample_008.fsa
sample_009.fsa
sample_010.fsa
sample_015.fsa
sample_016.fsa
sample_017.fsa
sample_018.fsa
sample_023.fsa
sample_024.fsa
sample_025.fsa
sample_026.fsa
sample_031.fsa
sample_032.fsa
sample_033.fsa
sample_034.fsa
sample_039.fsa
sample_040.fsa
sample_041.fsa
sample_042.fsa
SNPlex_48plex_v1
10
20
30
40
50
60
70
80
90
100
110
8,000
6,000
4,000
2,000
0

No.	Dye	Marker	Size	Left Range	Right Range	Allele Name	Control	Distance/kb	Comments
1	Blue	M5.1_001	12.6	0.5	0.5	A1	0	0.00	
2	Blue	M5.1_001	14.6	0.5	0.5	A2	0	0.00	
3	Blue	M5.1_002	15.9	0.5	0.5	A1	0	0.00	
4	Blue	M5.1_002	17.7	0.5	0.5	A2	0	0.00	
5	Blue	M5.1_003	20.0	0.5	0.5	A1	0	0.00	
6	Blue	M5.1_003	21.2	0.5	0.5	A2	0	0.00	
7	Blue	M5.1_004	22.5	0.5	0.5	A1	0	0.00	
8	Blue	M5.1_004	23.8	0.5	0.5	A2	0	0.00	
9	Blue	M5.1_005	25.1	0.5	0.5	A1	0	0.00	
10	Blue	M5.1_005	26.9	0.5	0.5	A2	0	0.00	
11	Blue	M5.1_CTL_001_	27.9	0.4	0.4	A1	0	0.00	
12	Blue	M5.1_CTL_001_	28.7	0.1	0.1	A2	0	0.00	
13	Blue	M5.1_006	29.6	0.5	0.5	A1	0	0.00	

```
[Panel Name]: SNPlex_48plex_v1
[Ploidy]: 2
```

Icons and Functions

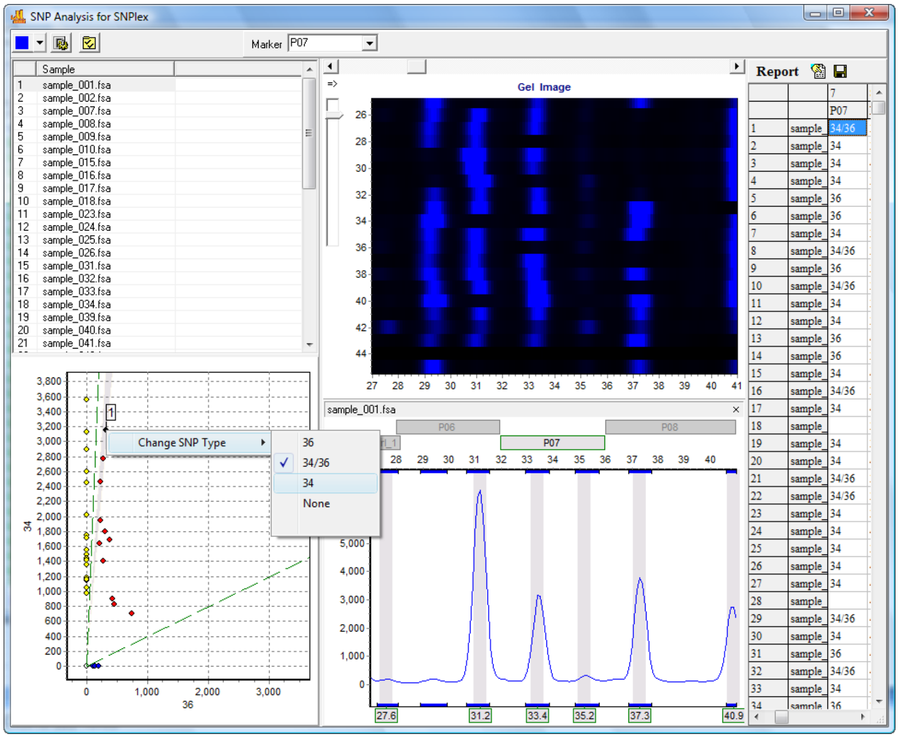
See *SNaPshot/SNuPE Icons and Functions* section above.

What to Expect

Since Peak Thresholds are not active in the Run Wizard when *SNPlex Analysis Type* is selected, the *SNP Analysis Settings* must be adjusted to correctly identify SNP peaks. Review the SNPlex results in the SNPlex Analysis module then click the **SNP Analysis Settings** icon. Enter a minimum threshold value in the *Min Intensity* field to remove low false-positive SNP calls. Optimize peak calls by setting the *Use Deviation when Intensity* value higher than the *Min Intensity* value but below the highest peak height in the dataset. Click **OK** in the *SNP Analysis*

Settings box and the thresholds will be applied. Use the Cartesian plot view to identify SNP calls in the gray region. Evaluate the SNP call and edit as necessary.

Editing SNP Calls



	Sample
1	sample_001.fsa
2	sample_002.fsa
3	sample_007.fsa
4	sample_008.fsa
5	sample_009.fsa
6	sample_010.fsa
7	sample_015.fsa
8	sample_016.fsa
9	sample_017.fsa
10	sample_018.fsa
11	sample_023.fsa
12	sample_024.fsa
13	sample_025.fsa
14	sample_026.fsa
15	sample_031.fsa
16	sample_032.fsa
17	sample_033.fsa
18	sample_034.fsa
19	sample_039.fsa
20	sample_040.fsa
21	sample_041.fsa

		7
		P07
1	sample_	34/36
2	sample_	34
3	sample_	34
4	sample_	34
5	sample_	36
6	sample_	36
7	sample_	34
8	sample_	34/36
9	sample_	36
10	sample_	34/36
11	sample_	34
12	sample_	34
13	sample_	36
14	sample_	36
15	sample_	34
16	sample_	34/36
17	sample_	34
18	sample_	
19	sample_	34
20	sample_	34
21	sample_	34/36
22	sample_	34/36
23	sample_	34
24	sample_	34
25	sample_	34
26	sample_	34
27	sample_	34
28	sample_	
29	sample_	34/36
30	sample_	34
31	sample_	36
32	sample_	34/36
33	sample_	34
34	sample_	36

SNPWave

One high-throughput method to determine SNP genotypes is SNPWave (Keygene N.V.). SNPWave uses multiplex oligonucleotide ligation amplification of allele-specific probes coupled with AFLP-primer selective amplification. SNPWave has the advantage of accurate high-throughput genotyping of up to 100 SNPs.

Circularizing padlock ligation probes are constructed that are specific to the SNP and flanking sequences. Locus-specific probes will hybridize to complementary denatured genomic DNA. Allele-specificity is determined by the SNP at the 5′ end of the padlock probe. Probes that contain a 5′ nucleotide complementary to the SNP will be ligated and amplified by PCR in subsequent reactions. Probes that do not contain a 5′ nucleotide complementary to the SNP will not be ligated and will not be amplified.

The padlock probes contain stuffer regions and primer binding sites for AFLP-selective amplification. The ligated padlock probe is amplified using fluorescently-labeled +2-selective and unlabeled non-selective AFLP primers. The stuffer region provides length discrimination between alleles and among loci. SNPs are separated by two basepairs and loci are separated by three basepairs. The fragments are separated by size using capillary electrophoresis. Fragment dye color and length indicate SNP locus and allele.

Analysis of SNPWave data follows the same procedure as SNPlex analysis. See *SNPlex* section above.

SNP Analysis Reporting

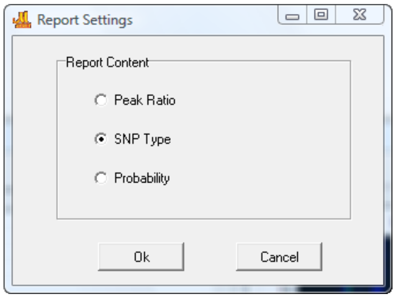
The *Report Table* in the *SNP Analysis* window, by default, displays the SNP type or alleles detected in the Marker. Click a cell in the *Report Table* to view the associated electropherogram.

Icons and Functions

The SNP type call can be modified in the *Report Table* by right-clicking a cell and selecting *Change SNP Type*. Select the correct SNP call from the fly-out menu and the *Report Table* will automatically update. Additionally, selecting Copy from the right-click menu will place the selected cell(s) information into the Windows clipboard which can be pasted into a spreadsheet program.

Report Settings

Launches the *Report Settings* box.



Report Content

○ Peak Ratio
● SNP Type
○ Probability

Peak Ratio

Calculates the ratio for the intensity of the first nucleotide divided by the intensity of the second nucleotide in a SNP pair.

SNP Type

Displays the nucleotide number of the peaks present in the SNP pair. *SNP Type* is the default view for the *Report Table*.

Probability

Displays the SNP types present and calculates a number between 0 and 1 for the likelihood that the SNP call is correct. The probability calculation takes into account the signal-to-noise ratio and peak intensities.

Save Report

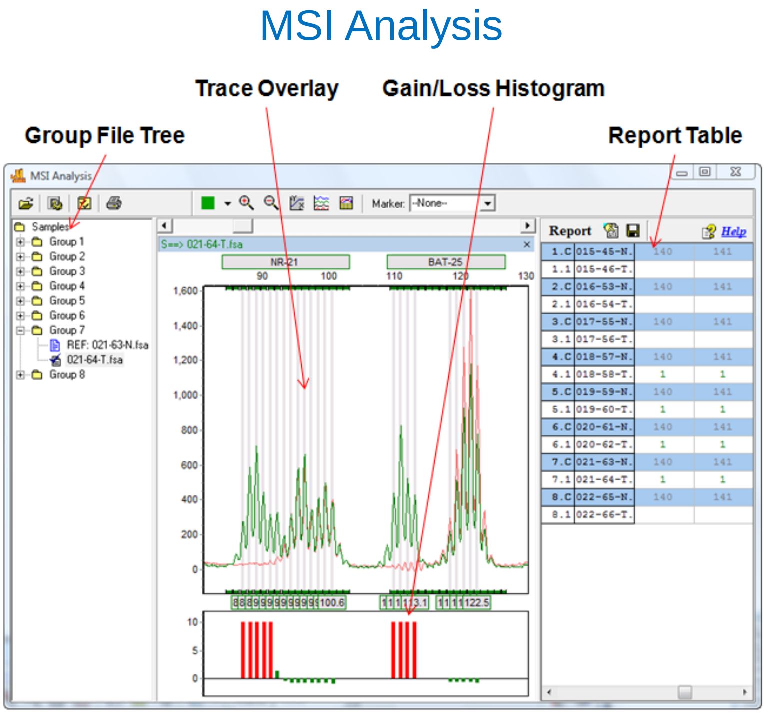
Saves the *Report Table* as an Excel (.xls) or tab-delimited Text (.txt) file.

Microsatellite Instability (MSI)

Microsatellites are stretches of DNA where a 1-5 base pair sequence is repeated several times. The most common microsatellite in humans is a dinucleotide repeat of CA which occurs tens of thousands of times across the genome. Microsatellite instability (MSI) is a condition where repeat units are gained or lost within a locus resulting in length polymorphism. Certain repeat regions are known to be highly polymorphic and hereditable. MSI is most frequently detected in regions with long mononucleotide repeats where DNA slippage during the polymerase reaction commonly occurs. Ultimately, microsatellite instability within and around certain genes can have devastating effects due to the possibility of frameshift mutations.

Overview

In GeneMarker's MSI Analysis module, tumor samples are compared to normal samples based on peak-to-peak comparison. Differences between the two traces are displayed in a *Gain/Loss Histogram* below each trace overlay. Within the electropherogram itself, the tumor sample trace is overlain on a light red trace of the reference. In this way, the clinician can easily visualize where the areas of instability exist.


MSI Analysis
Trace Overlay
Gain/Loss Histogram
Group File Tree
Report Table
MSI Analysis
Marker: --None--
Samples
Group 1
Group 2
Group 3
Group 4
Group 5
Group 6
Group 7
REF: 021-63-N.fsa
021-64-T.fsa
Group 8
S==> 021-64-T.fsa
NR-21
BAT-25
90
100
110
120
130
1,600
1,400
1,200
1,000
800
600
400
200
0
100.6
113.1
122.5
10
5
0
Report
Help

1.C	015-45-N.	140	141
1.1	015-46-T.		
2.C	016-53-N.	140	141
2.1	016-54-T.		
3.C	017-55-N.	140	141
3.1	017-56-T.		
4.C	018-57-N.	140	141
4.1	018-58-T.	1	1
5.C	019-59-N.	140	141
5.1	019-60-T.	1	1
6.C	020-61-N.	140	141
6.1	020-62-T.	1	1
7.C	021-63-N.	140	141
7.1	021-64-T.	1	1
8.C	022-65-N.	140	141
8.1	022-66-T.		

Group File Tree

Samples in the *Group File Tree* appear within Group folders according to the information provided in the *File Name Group Text* file uploaded in the *MSI Analysis Settings* box. A sample is marked as the Reference because it contains a *Control Identifier* as set in the *File Name Group* tool. **See Chapter 8 Additional Tools**. Reference/Control samples will appear in the first column of the *File Name Group Text* file.

Expand folders in the *Group File Tree* to view the Reference sample and Experimental sample in the group. Double-click the Reference sample to view just the Reference sample electropherogram. Double-click the Experimental sample to view the Experimental sample electropherogram overlaid on the Reference sample electropherogram and associated *Gain/Loss Histogram*. Use the **Up/Down Arrow** keys to navigate through samples. Hit the **Enter** key to open Group folders.

Right-click an Experimental sample in the *Group File Tree* and select *Set as Reference* to mark the sample as the Group's Reference sample.

Trace Overlay

The *Trace Overlay* displays the selected Experimental sample's trace superimposed over the selected Group's Reference trace. The Experimental sample trace line color will appear in the Marker's associated dye color. The Reference trace line color is displayed in light red. The Reference trace is, by default, drawn directly behind the Experimental sample trace. Click the **MSI Display Settings** icon to adjust the Reference trace offset as compared to the Experimental sample trace. Additionally, the RFU intensity or peak height of the Reference trace is normalized to the Experimental sample traces in the Group. To disable Reference trace normalization, click the **MSI Display Settings** icon and deselect *Peak Normalization*. The uncorrected Reference trace peak heights will be displayed in the *Trace Overlay*.

The allele editing options in the *Trace Overlay* frame are similar to those in the *Main Analysis* window *Electropherogram*. **See Chapter 3 Main Analysis Overview**.

Gain/Loss Histogram

The *Gain/Loss Histogram* indicates the degree to which the Experimental sample peak at a given position differs from the Reference sample peak at the same position based on *Peak Height* or *Peak Area* as set in the *MSI Analysis Settings* box. A Log2 function is applied to the *Peak Height/Area Ratio* to determine *MSI Score*. The *MSI Score* value is plotted in the *Gain/Loss Histogram*. Values above and below the minimum and maximum *MSI Score* thresholds set in the *MSI Analysis Settings* box will turn the Histogram bars red. The minimum and maximum *MSI Score* thresholds can therefore be used as a visual confidence level gauge in the *Gain/Loss Histogram*.

Report Table

The *Report Table* lists all samples in a column on the left. Reference samples are marked with a "C" which stands for "Control". In the Reference sample rows, which appear in blue, the basepair size positions of detected peaks are listed. In the Experimental sample rows, a "1", "0", "-1" appear indicating the Gain, Equivalent, or Loss, respectively, of a peak in the Experimental trace as compared to the Reference trace. Click the **Report Settings** icon and select/deselect to display Gain, Equivalent, or Loss values. Double-click values in the *Report Table* to link to the associated *Trace Overlay* position. Click the **Save Report** icon to save the *Report Table* as an Excel or tab-delimited Text file.

Procedure

1. Import MSI raw data files and filter with Run Wizard **Fragment (Animal) Analysis** settings. **See Chapter 2 General Procedure.**
2. Create a Panel for the data with the *Panel Editor* tool. **See Chapter 5 Panel Editor.**
3. Apply the Panel
4. Select *Applications → MSI Analysis*
5. The *MSI Analysis Settings* box appears
6. Upload a tab-delimited text file that identifies Reference samples (first column) and Experimental samples (second column) to the *Group File* field

NOTE: Use the *File Name Group Tool* to create a group file. **See Chapter 8 Additional Tools**.

7. Adjust *MSI Analysis Settings* as desired
8. Click **OK**
9. The *MSI Analysis* window appears
10. Click the **Print** icon to print the *MSI Clinical Report*

Icons and Functions

Load Group Information

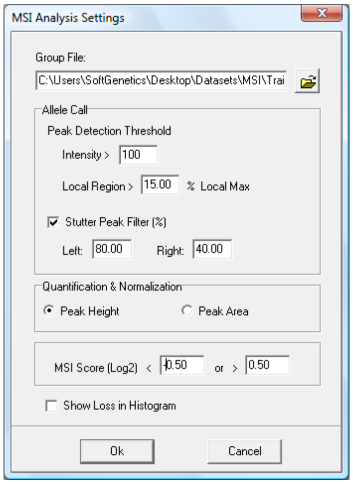
Choose a tab-delimited Text (.txt) file which contains information on how to group individual sample files. For example, pair Patient A's tumor sample to Patient A's normal sample by placing the normal sample filename in the first column and the tumor sample in the second column. **See Chapter 8 Additional Tools - Filename Group Tool.**

Analysis Settings

Opens the *MSI Analysis Settings* box.

Group File

Click *Open File* icon to upload a .txt file that groups samples by filename.



MSI Analysis Settings

Group File: C:\Users\SoftGenetics\Desktop\Datasets\MSI\Trai

Allele Call

Peak Detection Threshold

Intensity > 100

Local Region > 15.00 % Local Max

☑ Stutter Peak Filter (%)

Left: 80.00 Right: 40.00

Quantification & Normalization

● Peak Height ○ Peak Area

MSI Score (Log2) < -0.50 or > 0.50

☐ Show Loss in Histogram

Allele Call

Peak Detection Threshold

Intensity: Sets the minimum RFU intensity level at which peaks will be called.

Local Region: Sets the minimum percentage of the major peak in the dye color at which peaks will be called.

Stutter Peak Filter: Sets the minimum percentage of the major peak in a marker at which peaks to the right and left of the major peak will be called.

Quantification and Normalization

Peak Height: When selected, MSI Score and correcting for preferential amplification of smaller fragments (normalization) is calculated based on the RFU intensity values of a peak.

Peak Area: The same as Peak Height except calculations are based on the area under a peak.

MSI Score (Log2)

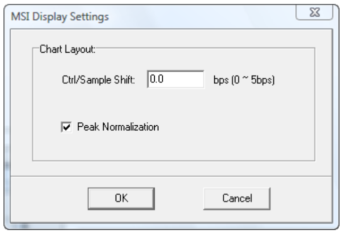
A logarithmic value, based on height or area, calculated for each individual peak. The *MSI Score* value is represented as a bar in the *Gain/Loss Histogram*. Setting the minimum and maximum values changes the range of score at which the peak will be considered "unstable". Confident MSI calls are shown as red bars in the Histogram, lesser confidence calls are displayed as green. The default settings will display both gains and losses (LOH) in the final report. Increase the negative value to -1.0 or more to filter out LOH reporting.

Show Loss in Histogram

When deselected only peaks with a positive MSI score will be represented with a bar in the histogram. Negative peaks (or losses) will not be shown.

Layout Settings

Launches the *MSI Display Settings* box.



MSI Display Settings

Chart Layout:

Ctrl/Sample Shift: 0.0 bps (0 ~ 5bps)

☑ Peak Normalization

Ctrl/Sample Shift

Change the amount of offset between the reference trace and the sample trace in the *Trace Overlay* electropherogram.

Peak Normalization

When selected, Reference sample peaks will be normalized based on peak height or area to the Experimental sample peaks in the Group.

Print

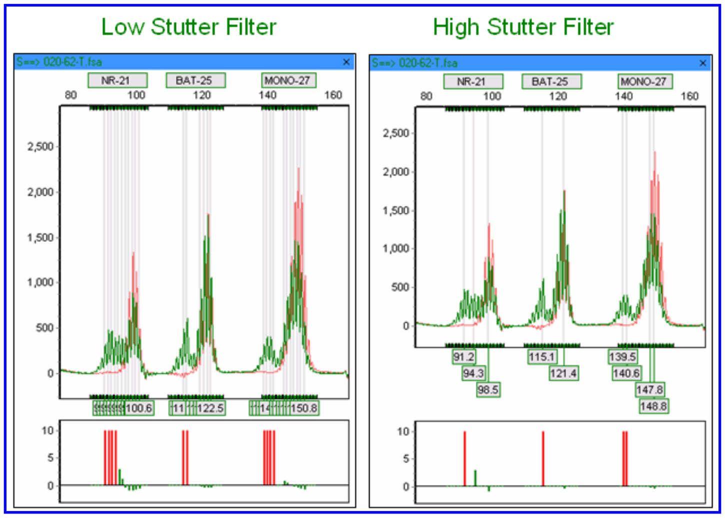
Launches the *MSI Print Settings* box. See *Reports and Printing* section below for more information.

What to Expect

There are a few options for MSI display which will vary by personal preference. In the MSI Analysis Settings box there are several options for filtering the allele call. The peak detection thresholds, Intensity and Local Region, are designed to filter out noise near the baseline. The Stutter Filter right and left thresholds are applied after the peak-to-peak comparison with the reference trace and are designed so the user can minimize the number of bars in the Peak Comparison Histogram. The image below shows a low stutter filter setting (Left: 60, Right: 40) compared to the same trace with a higher stutter filter setting applied (Left: 99, Right: 99).

Notice in the following example, when the stutter filter is decreased, the number of identified "instability" peaks increases, and vice versa for a higher stutter filter setting.

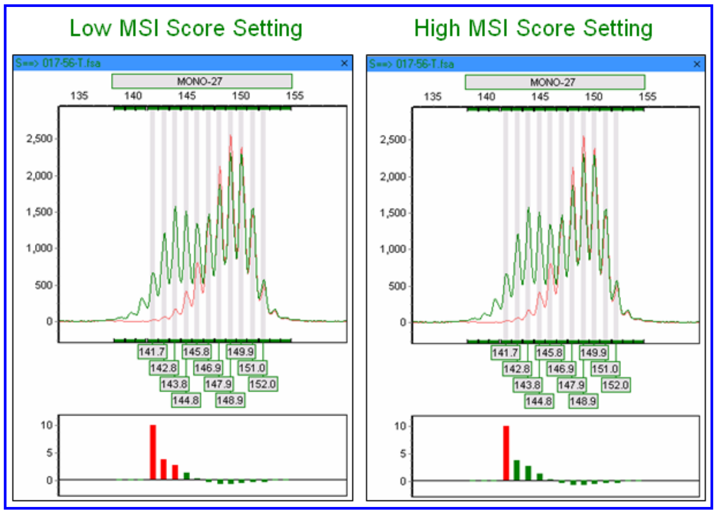
MSI Stutter Filter


Low Stutter Filter
High Stutter Filter
S==> 020-62-T.fsa
NR-21
BAT-25
MONO-27
80
100
120
140
160
2,500
2,000
1,500
1,000
500
0
100.6
122.5
150.8
91.2
94.3
98.5
115.1
121.4
139.5
140.6
147.8
148.8
10
5
0

A second option to help a clinician display only the most relevant peaks in a microsatellite instable marker is through the MSI Score setting. MSI Score is calculated using a $\log_2$ ratio plot of tumor sample versus reference. In this way, the normalized intensities of the reference sample can be accurately compared to the tumor sample thereby presenting a more meaningful analysis. To normalize the data, a single peak is divided by the average of all peak intensities across a dye color and the ratio is then used for peak-to-peak comparison. In the example below, a low MSI Score setting (-2, +2) is compared to a high MSI Score setting (-2, +4) for the same sample.

When the MSI Score threshold is lowered, more peaks are identified as unstable (red histogram bars). As the MSI Score threshold is increased, only the most relevant peaks are identified as instable.

MSI Score


Low MSI Score Setting
High MSI Score Setting
S==> 017-56-T.fsa
MONO-27
135
140
145
150
155
2,500
2,000
1,500
1,000
500
0
141.7
145.8
149.9
142.8
146.9
151.0
143.8
147.9
152.0
144.8
148.9
10
5
0

Reports and Printing

GeneMarker automates the analysis process and creates an easy-to-read report for fast MSI-High or Low determinations.

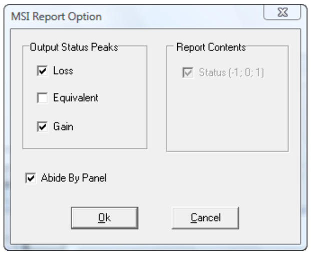
Report Table

Displays gain and loss information for individual peaks of a sample compared to a reference. Samples designated as “reference” by the filename group text file are marked as blue rows in the report while samples being compared to the reference are in the white rows. The *Overlay Trace* window will correspond to which ever peak cell is selected in the *Report Table*.

Report Settings
Launches the *MSI Report Options* dialog box.

Output Status Peaks
Choose to display Loss, Equivalent, or Gain peaks. Loss peaks are represented with a -1, Equivalent with a 0, and Gain with a 1. Peaks that do not meet the MSI Score thresholds for loss and gain will not be displayed in the Report Table.

Abide By Panel
When selected, all peaks regardless of their loss/gain status will be displayed. When deselected, only those peaks positions with a loss/gain will be displayed.


MSI Report Option
Output Status Peaks
☑ Loss
☐ Equivalent
☑ Gain
Report Contents
☑ Status (-1; 0; 1)
☑ Abide By Panel

Save Report
Choose to save the Report Table as an Excel file (.xls) or a tab-delimited Text file (.txt).

MSI Clinical Report Settings

Click the Print icon in the main tool bar to launch the *MSI Print Settings* box and preview, print or save the *MSI Analysis Report*.

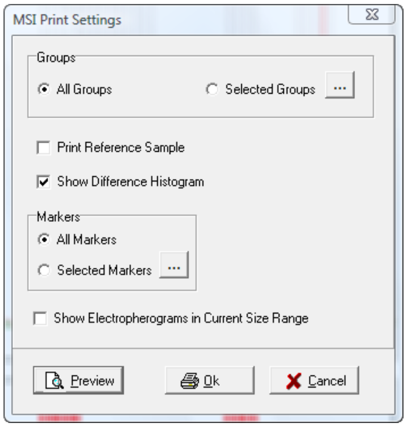
Groups
Choose *All Groups* or select specific groups to display in the report.

Print Reference Sample
When selected, a separate page with just the reference sample will be printed for each group.

Show Difference Histogram
When selected, the Gain/Loss Histogram will be displayed below each dye color's *Overlay Trace* electropherogram.

Markers
Choose All Markers or select specific markers to display in the report.

Show Electropherograms in Current Size Range
When selected, will display *Overlay Trace* electropherograms in the same zoom mode as the main *MSI Analysis* window. When deselected, the electropherograms will be displayed so that all selected markers can be clearly viewed.


MSI Print Settings
Groups
● All Groups ○ Selected Groups
☐ Print Reference Sample
☑ Show Difference Histogram
Markers
● All Markers
○ Selected Markers
☐ Show Electropherograms in Current Size Range

MSI Clinical Report

Below is a description of the MSI Clinical Report features. For an explanation of functions within the *Print Preview* window, see **Chapter 6 Reports and Printing.**

Report Header
Contains information about the analysis, project, sample and parameters.

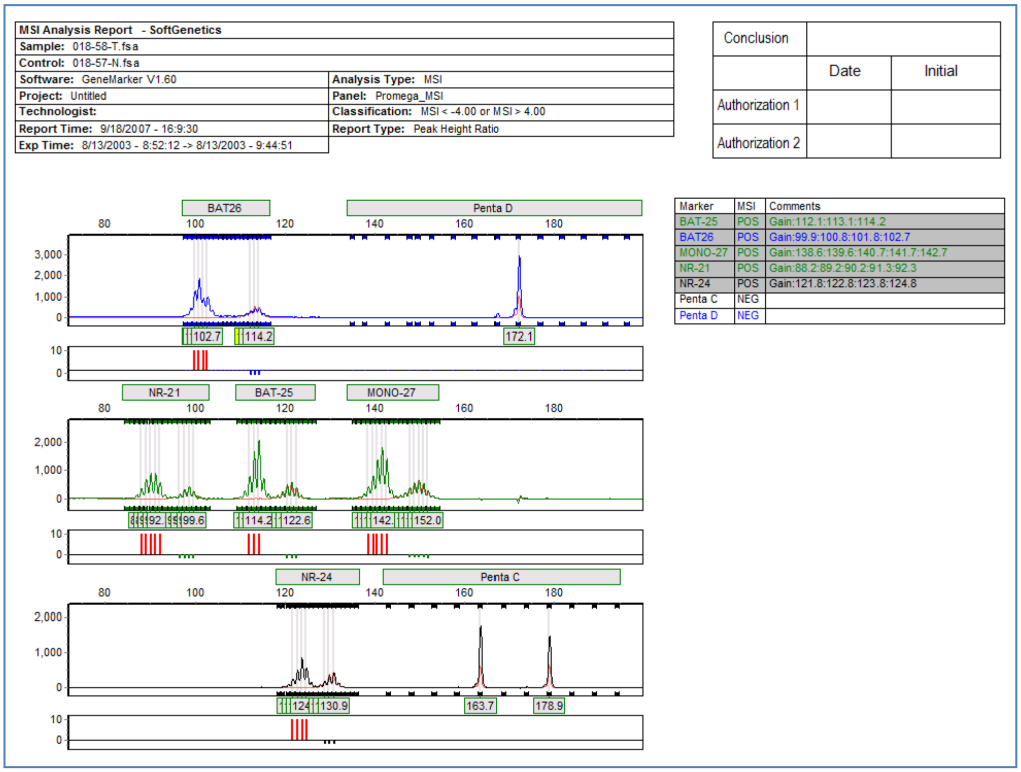
Signature Box
Date and initial space for report reviewers.

Overlay Trace Electropherogram
Similar to the main analysis window, displays the reference trace in light red behind the sample trace (dye color).

Gain/Loss Histogram
Also similar to the main analysis window, displays a histogram based on MSI Score and can be adjusted through the *MSI Analysis Settings* box.

Marker Table
Identifies the marker and whether it contains MSI (POS) or does not show MSI (NEG). The Comments column identifies the peak positions of the gains and losses (as compared to the reference).

MSI Clinical Report



MSI Analysis Report - SoftGenetics	
Sample: 018-58-T.fsa	
Control: 018-57-N.fsa	
Software: GeneMarker V1.60	**Analysis Type:** MSI
Project: Untitled	**Panel:** Promega_MSI
Technologist:	**Classification:** MSI < -4.00 or MSI > 4.00
Report Time: 9/18/2007 - 16:9:30	**Report Type:** Peak Height Ratio
Exp Time: 8/13/2003 - 8:52:12 -> 8/13/2003 - 9:44:51	

Conclusion		
	Date	Initial
Authorization 1		
Authorization 2		

Marker	MSI	Comments
BAT-25	POS	Gain:112.1:113.1:114.2
BAT26	POS	Gain:99.9:100.8:101.8:102.7
MONO-27	POS	Gain:138.6:139.6:140.7:141.7:142.7
NR-21	POS	Gain:88.2:89.2:90.2:91.3:92.3
NR-24	POS	Gain:121.8:122.8:123.8:124.8
Penta C	NEG	
Penta D	NEG	

Phylogeny Clustering Analysis

Biological applications of data clustering calculations include phylogeny analysis and community comparisons in ecology, gene expression pattern, enzymatic pathway mapping, and functional gene family classification in the bioinformatics field. It has been successfully paired with the AFLP analysis technique for a variety of applications.

There are two types of data clustering: hierarchical and partitional. Partitional clustering includes the K-means and Self-Organizing Map methods and will not be discussed here. Hierarchical clustering treats each data point as a single cluster and successively merges clusters until all points have been merged into a single remaining cluster. Hierarchical clustering is often represented as a dendrogram. In GeneMarker, the hierarchical algorithm is agglomerative and establishes clusters from the bottom up.

Overview

The first step in hierarchical clustering is to select a distance measure. GeneMarker distance options include *Euclidean Distance, Correlation Coefficient*, and *Percentage of Same Genotypes*. *Euclidean Distance* is the straight line distance between two points in two or three dimensional space. The equation is essentially the same as that for determining the length of the hypotenuse of a triangle – computed by finding the square of the distance between each variable, summing the squares, and finding the square root of that sum. We have simplified this equation (below) in GeneMarker. The *Correlation Coefficient* is based on the Pearson Correlation equation and is a statistical concept that quantifies the level of relationship between two sets of measurements. It is a measure of similarity where two values that are perfectly correlated have a distance of 1.00. *Percentage of Same Genotypes* is simply the number of similar genotypes divided by the total number of genotypes.

The following are GeneMarker's clustering algorithms:

Euclidean Distance

$$\sum_{i=1}^{n} \left|(x_i - y_i)\right|$$

Correlation Coefficient

$$r \equiv \sqrt{bb'} = \frac{n\sum xy - \sum x \sum y}{\sqrt{\left[n\sum x^2 - (\sum x)^2\right]\left[n\sum y^2 - (\sum y)^2\right]}}$$

In addition to a distance measure, the type of linkage needs to be applied. GeneMarker has three options: *Single, Complete,* and *Average* linkage. *Single linkage* measures the minimum distance between two clusters. Clustering using single linkage tends to produce an effect called chaining where single genes are added to clusters one at a time. *Complete linkage* is the opposite of single linkage. It measures the distance between the farthest two points in the clusters. Complete linkage performs well when the clusters are well defined with minimal noise. *Average linkage* defines the distance between two clusters as the mean distance between all points in the clusters.
NOTE: Choosing different linkage measures results in different cluster diagrams.

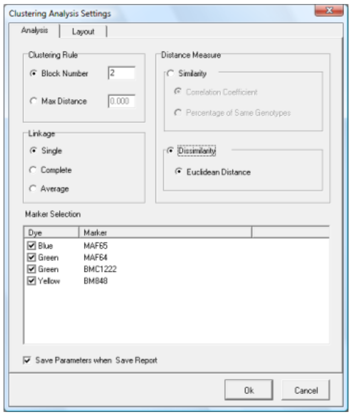
Procedure

1. Upload and filter the data with Run Wizard. **See Chapter 2 General Procedure**.
2. Apply a Panel to the dataset. **See Chapter 5 Panel Editor**.
3. Select *Applications* → *Clustering* Analysis
4. The *Clustering Analysis* module will appear
5. Click the **Clustering Analysis Settings** icon
6. The *Clustering Analysis Settings* box will appear
7. Adjust settings
8. Click **OK**
9. Click the **Save Dendrogram** icon to save the image as a BMP file
10. Click the **Output Clustering Report** icon to save the matrix values as a tab-delimited Text file

Icons and Functions

Clustering Analysis Settings

Select this option to choose between two tabs: Analysis and Layout



Clustering Rule
● Block Number 2
○ Max Distance 0.000

Linkage
● Single
○ Complete
○ Average

Distance Measure
○ Similarity
● Correlation Coefficient
○ Percentage of Same Genotypes
● Dissimilarity
● Euclidean Distance

Marker Selection

Dye	Marker
☑ Blue	MAF65
☑ Green	MAF64
☑ Green	BMC1222
☑ Yellow	BM848

☑ Save Parameters when Save Report

Analysis

The *Analysis* tab is used to set cluster calculation parameters.

Clustering Rule

Block Number – Choose the number of expected major groups in the dendrogram

Max Distance/Min Correlation – Choose the distance or correlation percentage at which to start the grouping of individuals

Distance Measure

See *Overview* section above for definitions of Correlation Coefficient, Percentage of Same Genotypes, and Euclidian Distance.

Linkage

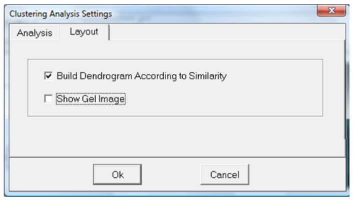
See *Overview* section above for definitions of Single, Complete, and Average linkage.

Marker Selection

Dye/Marker – choose which Markers to include in the clustering calculation and dendrogram
Save Parameters when Save Report – to automatically create an .ini file of the AnalysisSettings

Layout

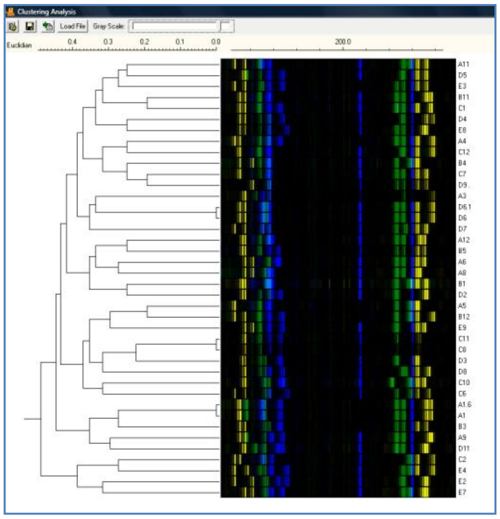
Build Dendrogram According to Similarity When selected, will show the distance/correlation ruler above the dendrogram tree. When deselected the distance/correlation ruler will be removed.

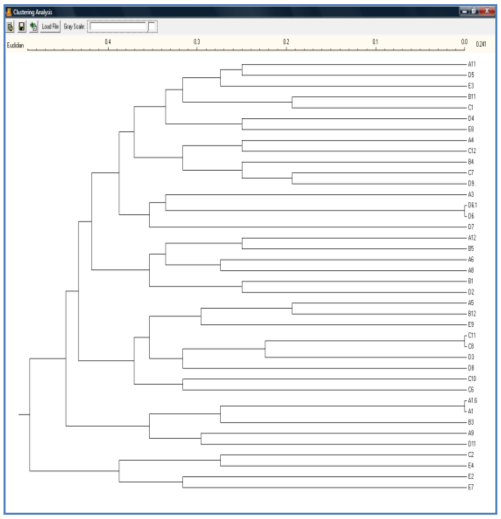

Clustering Analysis Settings

Analysis | Layout

☑ Build Dendrogram According to Similarity
☐ Show Gel Image

Show Gel Image provides flexibility of hiding or showing the gel image in the dendogram


Clustering Analysis
Load File
Gray Scale
Euclidian
0.4
0.3
0.2
0.1
0.0
200.0


Clustering Analysis
Load File
Gray Scale
Euclidian
0.4
0.3
0.2
0.1
0.0

Save Dendrogram

Allows the user to save the dendrogram image as a BMP image file or as a PNG file. PNG uses lossless compression leading to greatly reduced image size.

Output Clustering Report

Produces a matrix table that can be saved as a Text (.txt) file. Choose the analysis method from the drop-down icon in the main toolbar.

What to Expect

Notice how when just the distance measure is changed (Fig 1 & 2), the basic overall structure is similar, however; on closer examination the fine structure of ordering within the main clusters differs. The samples with "3" as the first character are grouped, as are the samples with the number "4". The sole "7" sample is grouped in its own cluster in both examples. These results are as expected. Figure 1 and 2 also show an example of isomorphism in the dendrogram, where the "3" group and "4" group positions are switched.

Fig. 1 Euclidean Distance Single Linkage

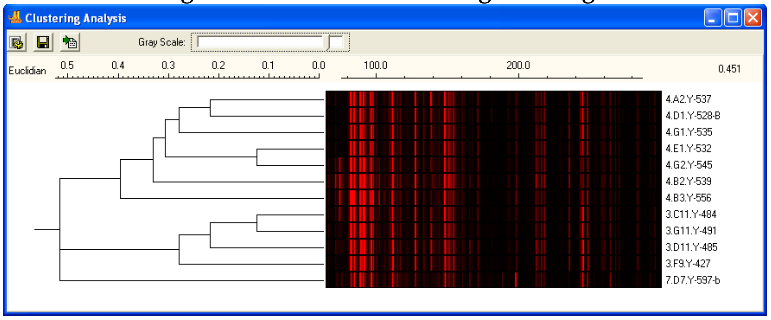
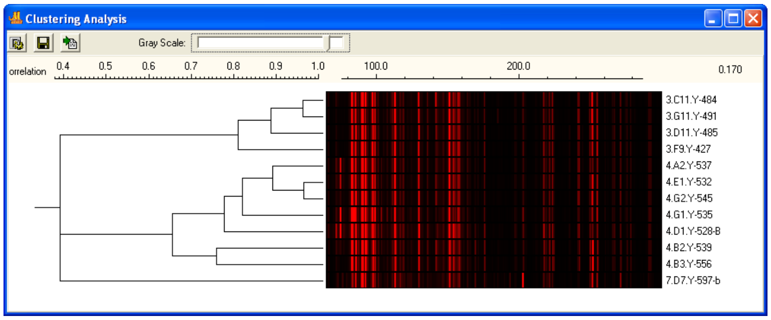

Clustering Analysis
Gray Scale:
Euclidian
0.5
0.4
0.3
0.2
0.1
0.0
100.0
200.0
0.451
4.A2.Y-537
4.D1.Y-528-B
4.G1.Y-535
4.E1.Y-532
4.G2.Y-545
4.B2.Y-539
4.B3.Y-556
3.C11.Y-484
3.G11.Y-491
3.D11.Y-485
3.F9.Y-427
7.D7.Y-597-b

Fig. 2 Correlation Coefficient Single Linkage


Clustering Analysis
Gray Scale:
orrelation
0.4
0.5
0.6
0.7
0.8
0.9
1.0
100.0
200.0
0.170
3.C11.Y-484
3.G11.Y-491
3.D11.Y-485
3.F9.Y-427
4.A2.Y-537
4.E1.Y-532
4.G2.Y-545
4.G1.Y-535
4.D1.Y-528-B
4.B2.Y-539
4.B3.Y-556
7.D7.Y-597-b

When altering the analysis based just on linkage type and holding the distance measure constant (Fig 2-4), we see that the overall structure remains the same, however; the finer structure is greatly affected. Notice how in single linkage (Fig 2) the three main groups are independent of one another where in complete (Fig3) and average linkage(Fig 4), the "3" group and "4" group are derived from the same cluster. It can also be seen from this example how average linkage is an amalgam of single and complete linkage.

Fig. 3 Correlation Coefficient Complete Linkage

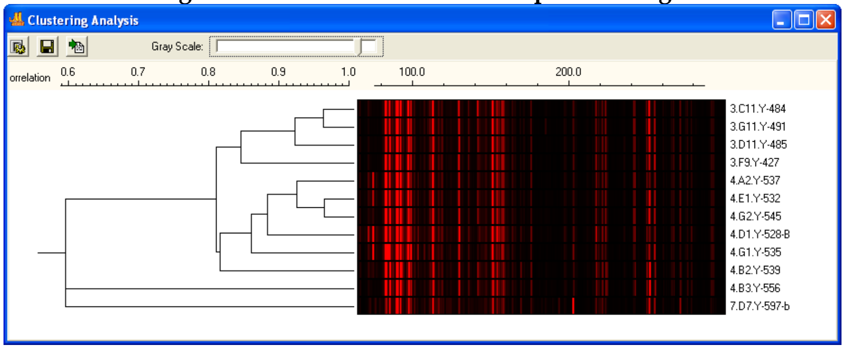
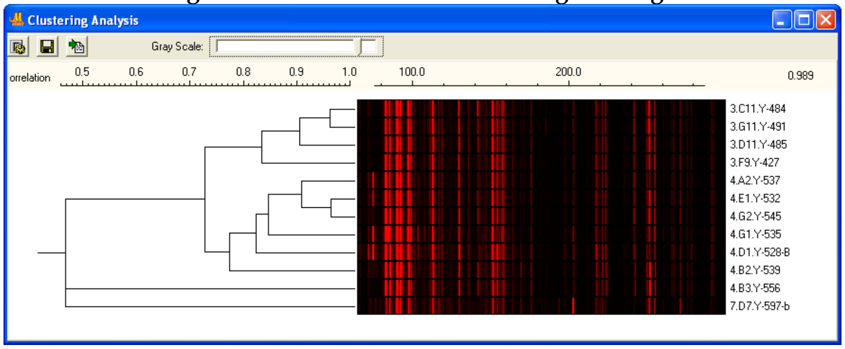
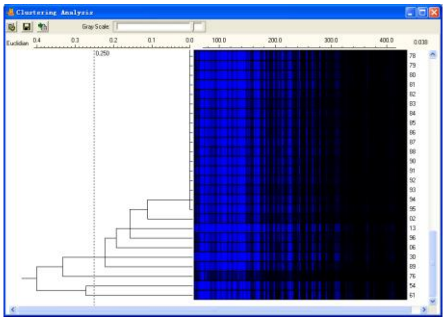

Clustering Analysis
Gray Scale:
orrelation
0.6
0.7
0.8
0.9
1.0
100.0
200.0
3.C11.Y-484
3.G11.Y-491
3.D11.Y-485
3.F9.Y-427
4.A2.Y-537
4.E1.Y-532
4.G2.Y-545
4.D1.Y-528-B
4.G1.Y-535
4.B2.Y-539
4.B3.Y-556
7.D7.Y-597-b

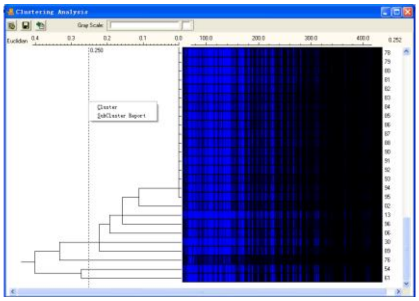
Fig. 4 Correlation Coefficient Average Linkage


Clustering Analysis
Gray Scale:
orrelation
0.5
0.6
0.7
0.8
0.9
1.0
100.0
200.0
0.989
3.C11.Y-484
3.G11.Y-491
3.D11.Y-485
3.F9.Y-427
4.A2.Y-537
4.E1.Y-532
4.G2.Y-545
4.G1.Y-535
4.D1.Y-528-B
4.B2.Y-539
4.B3.Y-556
7.D7.Y-597-b

Detecting Euclidian Distance

Press Ctrl and click anywhere in the linkage chart under the scale, a dashed vertical line will show up indicating that distance.



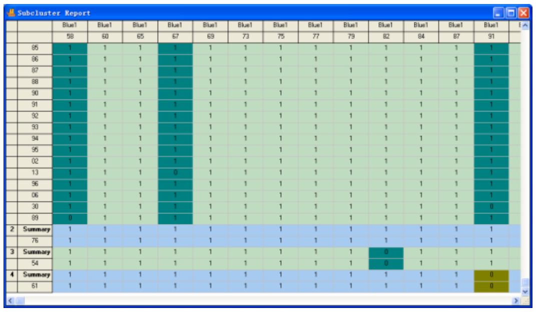


Sub-cluster Report and Saving

Right click to obtain a pop up menu. Left click to select SubCluster Report.

The table shows the grouping at that particular distance as well as the presence or absence of each allele for each individual sample, with 1 indicating presence and 0 indicating absence. The summary row of each group indicates the consistence or inconsistence at each allele for all individual samples in that group, with 1 indicating consistence and 0 indicating inconsistence. If all individual samples have value 1 at a certain allele, then they are consistent. Otherwise they are not. Different background colors are used to make a distinctive look.

Left click to pop up a Save As dialog box and save this table to a txt file with specified name.

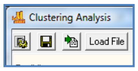


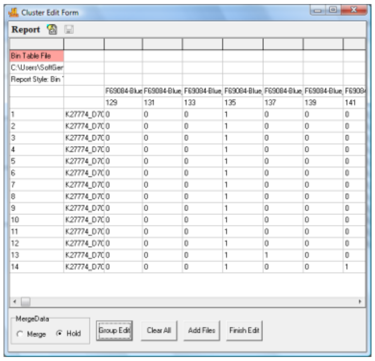
		Blue1	Blue1	Blue1	Blue1	Blue1	Blue1	Blue1	Blue1	Blue1	Blue1	Blue1	Blue1	Blue1
		58	60	65	67	69	73	75	77	79	82	84	87	91
	85	1	1	1	1	1	1	1	1	1	1	1	1	1
	86	1	1	1	1	1	1	1	1	1	1	1	1	1
	87	1	1	1	1	1	1	1	1	1	1	1	1	1
	88	1	1	1	1	1	1	1	1	1	1	1	1	1
	90	1	1	1	1	1	1	1	1	1	1	1	1	1
	91	1	1	1	1	1	1	1	1	1	1	1	1	1
	92	1	1	1	1	1	1	1	1	1	1	1	1	1
	93	1	1	1	1	1	1	1	1	1	1	1	1	1
	94	1	1	1	1	1	1	1	1	1	1	1	1	1
	95	1	1	1	1	1	1	1	1	1	1	1	1	1
	02	1	1	1	1	1	1	1	1	1	1	1	1	1
	13	1	1	1	0	1	1	1	1	1	1	1	1	1
	96	1	1	1	1	1	1	1	1	1	1	1	1	1
	06	1	1	1	1	1	1	1	1	1	1	1	1	1
	30	1	1	1	1	1	1	1	1	1	1	1	1	0
	89	0	1	1	1	1	1	1	1	1	1	1	1	1
2	Summary	1	1	1	1	1	1	1	1	1	1	1	1	1
	76	1	1	1	1	1	1	1	1	1	1	1	1	1
3	Summary	1	1	1	1	1	1	1	1	1	0	1	1	1
	54	1	1	1	1	1	1	1	1	1	0	1	1	1
4	Summary	1	1	1	1	1	1	1	1	1	1	1	1	0
	61	1	1	1	1	1	1	1	1	1	1	1	1	0

Using Allele Bin Report from Merged Projects

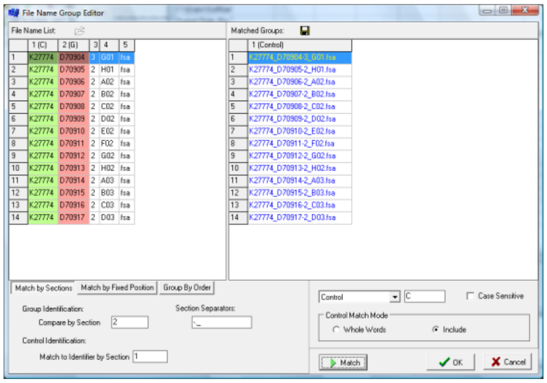
A common challenge with cluster analysis in animal and plant populations is obtaining enough informative markers in one multiplex. Overlapping marker ranges and/or incompatible chemistry make it necessary to run the same samples multiple times with different sets of marker primers. Cluster analysis using the combined results of two or more multiplexes (or kits).

1. Select Load File → Add Files and navigate to the saved merged bin report (Please see Merge Projects, Chapter 8)
2. Select Group Edit →Enter values for Group Identification and Control Identification
3. Select Match → OK
4. Select Finish Edit to display the Cluster Analysis of the combined project results





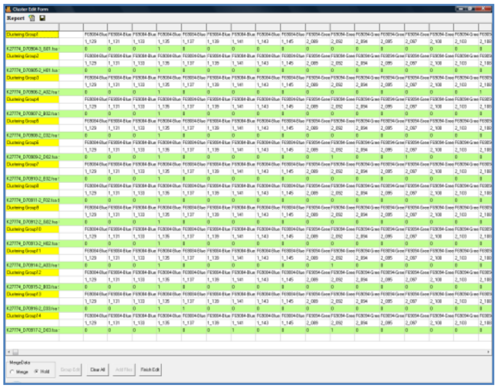
Bin Table File								
C:\Users\SoftGer								
Report Style: Bin								
		F69084-Blue	F69084-Blue	F69084-Blue	F69084-Blue	F69084-Blue	F69084-Blue	F6908
		129	131	133	135	137	139	141
1	K27774_D70	0	0	0	1	0	0	0
2	K27774_D70	0	0	0	1	0	0	0
3	K27774_D70	0	0	0	1	0	0	0
4	K27774_D70	0	0	0	1	0	0	0
5	K27774_D70	0	0	0	1	0	0	0
6	K27774_D70	0	0	0	1	0	0	0
7	K27774_D70	0	0	0	1	0	0	0
8	K27774_D70	0	0	0	1	0	0	0
9	K27774_D70	0	0	0	1	0	0	0
10	K27774_D70	0	0	0	1	0	0	0
11	K27774_D70	0	0	0	1	0	0	0
12	K27774_D70	0	0	0	1	0	0	0
13	K27774_D70	0	0	0	1	1	0	0
14	K27774_D70	0	0	0	1	0	0	1



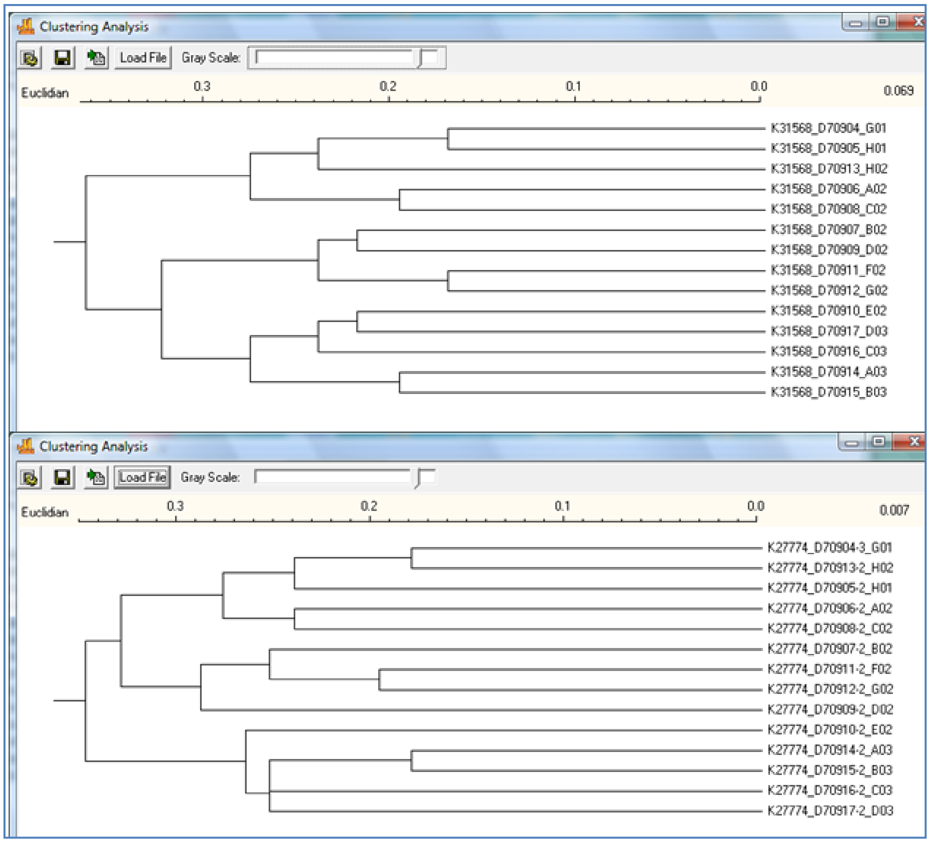
	1 (C)	2 (G)	3	4	5
1	K27774	D70904	3	G01	fsa
2	K27774	D70905	2	H01	fsa
3	K27774	D70906	2	A02	fsa
4	K27774	D70907	2	B02	fsa
5	K27774	D70908	2	C02	fsa
6	K27774	D70909	2	D02	fsa
7	K27774	D70910	2	E02	fsa
8	K27774	D70911	2	F02	fsa
9	K27774	D70912	2	G02	fsa
10	K27774	D70913	2	H02	fsa
11	K27774	D70914	2	A03	fsa
12	K27774	D70915	2	B03	fsa
13	K27774	D70916	2	C03	fsa
14	K27774	D70917	2	D03	fsa

	1 (Control)
1	K27774_D70904-3_G01.fsa
2	K27774_D70905-2_H01.fsa
3	K27774_D70906-2_A02.fsa
4	K27774_D70907-2_B02.fsa
5	K27774_D70908-2_C02.fsa
6	K27774_D70909-2_D02.fsa
7	K27774_D70910-2_E02.fsa
8	K27774_D70911-2_F02.fsa
9	K27774_D70912-2_G02.fsa
10	K27774_D70913-2_H02.fsa
11	K27774_D70914-2_A03.fsa
12	K27774_D70915-2_B03.fsa
13	K27774_D70916-2_C03.fsa
14	K27774_D70917-2_D03.fsa

Group Identification:
Compare by Section: 2
Section Separators: -_
Control Identification:
Match to Identifier by Section: 1
Control: C
☐ Case Sensitive
○ Whole Words ● Include



What To Expect


Clustering Analysis
Load File
Gray Scale:
Euclidian
0.3
0.2
0.1
0.0
0.069
K31568_D70904_G01
K31568_D70905_H01
K31568_D70913_H02
K31568_D70906_A02
K31568_D70908_C02
K31568_D70907_B02
K31568_D70909_D02
K31568_D70911_F02
K31568_D70912_G02
K31568_D70910_E02
K31568_D70917_D03
K31568_D70916_C03
K31568_D70914_A03
K31568_D70915_B03
Clustering Analysis
Load File
Gray Scale:
Euclidian
0.3
0.2
0.1
0.0
0.007
K27774_D70904-3_G01
K27774_D70913-2_H02
K27774_D70905-2_H01
K27774_D70906-2_A02
K27774_D70908-2_C02
K27774_D70907-2_B02
K27774_D70911-2_F02
K27774_D70912-2_G02
K27774_D70909-2_D02
K27774_D70910-2_E02
K27774_D70914-2_A03
K27774_D70915-2_B03
K27774_D70916-2_C03
K27774_D70917-2_D03

The analysis in the upper diagram is based on just the results from the 4 marker multiplex. The analysis in the lower diagram is from the combined allele reports.

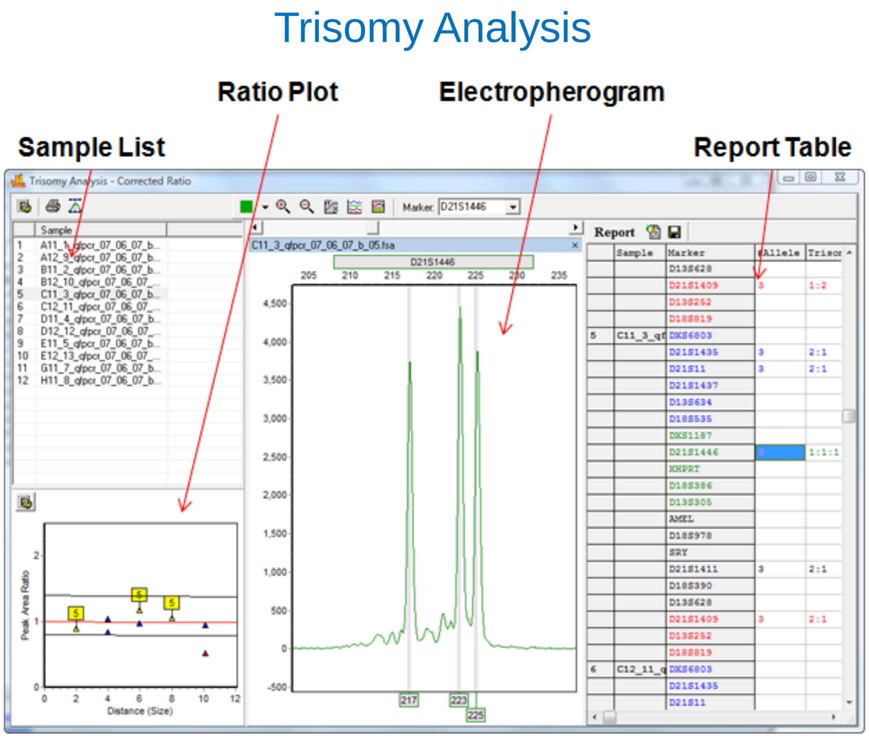
Trisomy Detection

Full trisomy of an individual occurs due to non-disjunction during meiosis I or meiosis II of gametogenesis resulting in 24 vice 23 chromosomes in a reproductive cell (sperm or egg). Thus, after fertilization, the resulting fetus has 47 chromosomes vice the typical 46. The most common forms of autosomal trisomy are trisomy of chromosome 21 which results in Down Syndrome and trisomy of chromosome 18 which results in Edwards Syndrome. In rare cases, a fetus with trisomy of chromosome 13 can survive. Trisomy 13 is called Patau Syndrome. Autosomal trisomy is frequently associated with severe congenital abnormalities, mental retardation and shortened life expectancy. Aneuploidy of sex chromosomes can also occur: The presence of extra X chromosome(s) causes Klinefelter syndrome in men and Triple X syndrome in women, while monosomy X (45, X) gives rise to women with Turner syndrome.

GeneMarker is a software tool used in clinical diagnostics and research laboratories across the world to analyze DNA fragments. GeneMarker's new Trisomy detection module aids clinicians and researchers in analyzing QF-PCR products to detect aneuploidy.

Overview

In GeneMarker's Trisomy Analysis module, the ratio of peaks within a marker is calculated. Markers which contain three peaks are obviously trisomy; however, markers which contain two peaks in a 1:2 or 2:1 ratio are more difficult to detect. Given user defined thresholds, trisomy peaks in a 1:2 or 2:1 ratio appear as red triangles in the *Ratio Plot* and appear in dye-colored font in the *Report Table*. In this way, the clinician can easily identify markers which contain trisomy peaks.



	Sample
1	A11_1_qfpcr_07_06_07_b...
2	A12_9_qfpcr_07_06_07_b...
3	B11_2_qfpcr_07_06_07_b...
4	B12_10_qfpcr_07_06_07_...
5	C11_3_qfpcr_07_06_07_b...
6	C12_11_qfpcr_07_06_07_...
7	D11_4_qfpcr_07_06_07_b...
8	D12_12_qfpcr_07_06_07_...
9	E11_5_qfpcr_07_06_07_b...
10	E12_13_qfpcr_07_06_07_...
11	G11_7_qfpcr_07_06_07_b...
12	H11_8_qfpcr_07_06_07_b...

	Sample	Marker	#Allele	Triso
		D13S628		
		D21S1409	3	1:2
		D13S252		
		D18S819		
5	C11_3_qf	DXS6803		
		D21S1435	3	2:1
		D21S11	3	2:1
		D21S1437		
		D13S634		
		D18S535		
		DXS1187		
		D21S1446	3	1:1:1
		XHPRT		
		D18S386		
		D13S305		
		AMEL		
		D18S978		
		SRY		
		D21S1411	3	2:1
		D18S390		
		D13S628		
		D21S1409	3	2:1
		D13S252		
		D18S819		
6	C12_11_q	DXS6803		
		D21S1435		
		D21S11		

Sample List

The *Sample List* displays all current samples in the project. Single left-click a sample name or use the **Up/Down Arrow** keys to scroll through the list.

Electropherogram

The *Electropherogram* shows the trace for the sample by marker. Select a marker to view from the Marker drop-down menu in the main toolbar.

The allele editing options in the *Electropherogram* frame are similar to those in the *Main Analysis* window. **See Chapter 3 Main Analysis Overview**.

Ratio Plot

The *Ratio Plot* depicts data points representing Marker peak ratios on a peak ratio vs. basepair size graph. Markers with three peaks will display three data points, one for each peak. The three data points will typically appear within the user-defined ratio threshold limits (black lines) and along the red fit line. Markers with two peaks are represented by only one data point in the *Ratio Plot*. If the data point is a blue triangle, then the ratio of the two peaks is within the user-defined ratio threshold limits. If the data point is a red triangle, then the ratio of the two peaks is outside the user-defined ratio threshold limits and will be considered trisomy. Data points that approach the black limit lines need to be considered carefully as they are not in perfect 1:2 (0.5) or 2:1 (2.0) ratio. **NOTE:** Markers which contain a single peak (homozygote) are not represented in the *Ratio Plot*.

Single-click data points in the *Ratio Plot* to view the Marker in the *Electropherogram* and the *Report Table* values. To zoom in, hold down left-click and drag a box from upper left to lower right. To zoom out, hold down left-click and drag a box in the opposite direction from lower right to upper left.

Click the **Settings** icon in the *Ratio Plot*. The *Trisomy Marker Statistic Settings* box appears. Adjust the settings to display the *Ratio Plot* as a direct ratio calculation or as the corrected ratio calculation plot. Change the coefficient value to adjust the slope of the fit line.

Report Table

The *Report Table* lists all samples in a column on the left. Selected report values appear in columns along the top. Click the **Report Settings** icon and select/deselect reported values. Double-click values in the *Report Table* to link to the associated *Electropherogram* position. Click the **Save Report** icon to save the *Report Table* as an Excel or tab-delimited Text file. See the *Reports and Printing* section below for more *Report Table* features.

Procedure

1. Open GeneMarker and upload raw data files
2. Select a Panel, Size Standard and **Fragment (Animal) Analysis**
3. Verify allele calls are accurate in the *Main Analysis* window and adjust parameter settings accordingly. **See Chapter 2 General Procedure.**
4. In the **Applications** menu, select **Trisomy Analysis**
5. The *Trisomy Analysis Settings* box appears
6. Adjust settings and click **OK**
7. The *Trisomy Analysis* window appears

Icons and Functions

Trisomy Analysis Settings

Opens the *Trisomy Analysis Settings* box. Two tabs are available: *Analysis* tab and *Statistics Plot* tab.

Analysis Tab

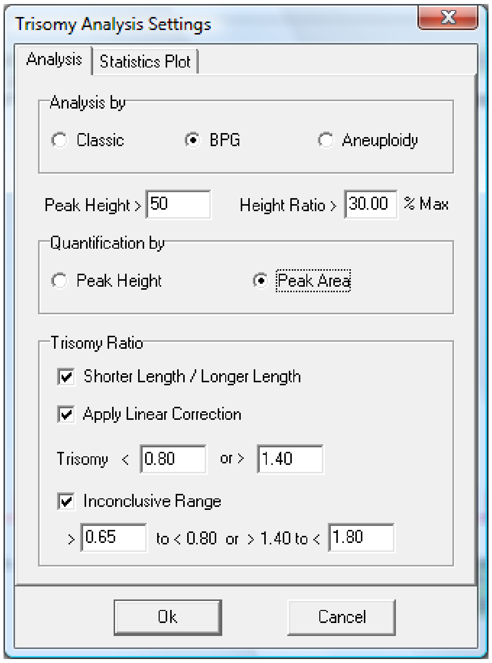
Provides threshold setting options for Trisomy analysis. To change default settings enter the desired ranges in the dialog box.

Analysis

Classic: When selected, Quantification is by Peak Height and Trisomy Ratio default thresholds are <0.70 or > 1.50. Additionally, the *Report Table* in the Trisomy Analysis window will display *Trisomy Score* values and the *Trisomy Print Report* will be set to *Classic* view. See the *Reports and Printing* section below.

BPG: When selected, Quantification is by Peak Area and Trisomy Ratio default thresholds are <0.80 or > 1.40. Additionally, the *Report Table* in the Trisomy Analysis window will display *Peak Ratio* values and the *Trisomy Print Report* will be set to *BPG* view. See the *Reports and Printing* section below.

Aneuploidy: When selected, Quantification is by peak area and default thresholds are <0.80 or > 1.40. Select



Analysis by
○ Classic ● BPG ○ Aneuploidy

Peak Height > 50
Height Ratio > 30.00 % Max

Quantification by
○ Peak Height ● Peak Area

Trisomy Ratio
☑ Shorter Length / Longer Length
☑ Apply Linear Correction
Trisomy < 0.80 or > 1.40
☑ Inconclusive Range
> 0.65 to < 0.80 or > 1.40 to < 1.80

Shorter Length/Longer Length. The report table is similar to BPG with ability to report results on sex chromosomes as well as autosomes.

Thresholds

Peak Height: Minimum height a peak must reach to be called.

Height Ratio: Maximum percentage of the main peak the second peak must reach in order for two alleles to be identified.

Quantification

Peak Height: Ratio calculations are generated based on the RFU values of two peaks within a Marker.

Peak Area: Ratio calculations are generated based on the area values of two peaks within a Marker.

Trisomy Ratio

Apply Linear Correction: When selected, ratio values will be corrected for based on the slope of peak heights within a Marker. The slope of all sample peaks within a single Marker is represented by the red *Fit Line* in the *Ratio Plot*. Deselect this option to view raw, uncorrected peak height or area ratios.

Trisomy: Set lower and upper limits for Trisomy detection. The values entered here are represented by the black lines in the *Ratio Plot*.

Inconclusive Range: Samples that fall within an incolnclusive range will be identified by a ? in the report table if this option is selected, as specified by the Best Practice Guidelines (2007)

Statistics Plot Tab

Provides display options for the *Ratio Plot*.

Coordinate Y

Peak Ratio: Evaluates trisomy based on differences in intensity of peaks within a Marker.

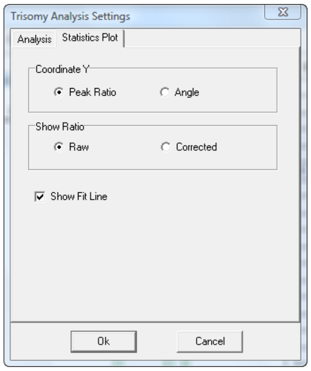
Angle: Evaluates trisomy based on the angle of a line that is formed between the center of the tops of the peaks in a Marker.

Show Ratio

Raw: Calculates the ratio of peak intensities based on raw data.

Corrected: Calculates the ratio of peak intensities based on the normalized data. Normalization is the process by which large fragment's relatively lower intensities are increased to equal small fragment intensity using a linear function.

Show Fit Line: When selected, a red center line is placed between the trisomy detection limits in the *Ratio Plot*. The Fit Line represents the slope of the peak heights for all the samples in the dataset at the selected Marker.



Coordinate Y: ● Peak Ratio ○ Angle

Show Ratio: ● Raw ○ Corrected

☑ Show Fit Line

Print Report

Launches the *Trisomy Print Settings* box. See *Reports and Printing* section below for more information.

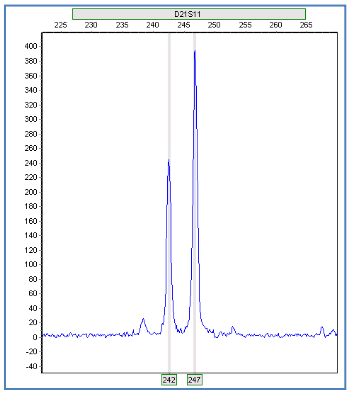
Show Bins

Click the *Show Bins* icon to display Panel Bins in the *Electropherogram*. The *Show Bins* option selected when the *Print* icon is clicked is the setting that will be applied to the *Trisomy Print Report*.

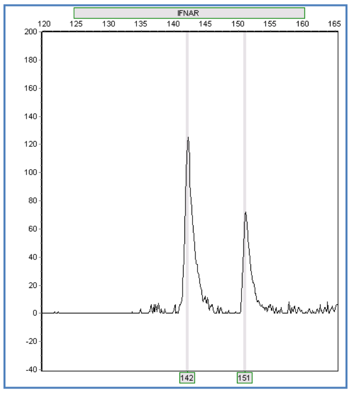
What to Expect

GeneMarker has been designed to accurately detect aneuploidy using short tandem repeat markers derived from PCR DNA fragments. Trisomy individuals will either show three fragments of equal intensity or two fragments at a 2:1 or 1:2 ratio.

Ratio 1:2



Ratio 2:1



Ratio 1:2 & 2:1 Trisomy

When DNA fragments are run through a PCR reaction, the smaller fragments are preferentially amplified. Electrophoresis injection also holds a bias toward smaller fragments. Subsequently, the smaller fragments' peak intensity in an electropherogram will be higher than the larger fragments in the sample. This is called preferential amplification and it is important in trisomy detection; especially, allele ratios that are 1:2 or 2:1. The 1:2 allele ratio occurs when the individual has 1 allele in the first position and 2 alleles in the second position in a marker; vice versa for a 2:1 ratio. So the question arises; is this ratio imbalance real or is it due to preferential amplification of the first allele?

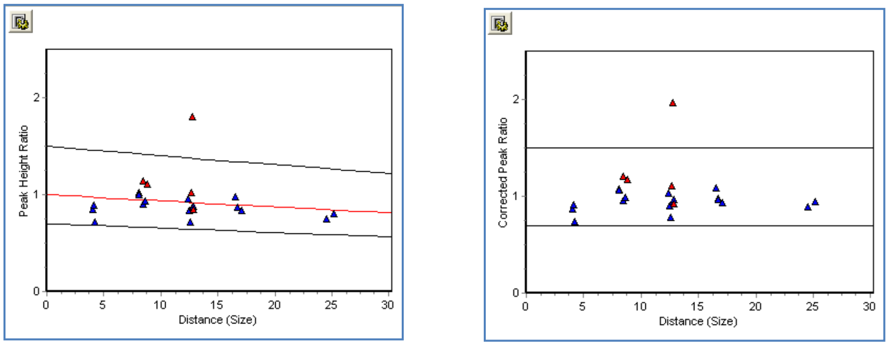
Ratio Plot

The GeneMarker Trisomy tool offers two answers to the 1:2/2:1 trisomy detection question. First, in the *Ratio Plot* in the bottom left corner of the analysis window, the peak intensity ratio of all markers are plotted. A linear regression line is run through the center of the data points and is used to correct for intensity drop due to fragment size increase. The *Ratio Plot* can be viewed as a linear regression plot or corrected for slope. This method of data correction aids in the detection of imbalanced ratio trisomy.

T-Score

The second aid in trisomy determination is the trisomy score. First a t-value is determined and defined as the difference between the sample and the expected value divided by the standard deviation. There are two possible t-values for every marker, one is the t-value for heterozygote and the second is for a trisomy. T-Score is the ratio of the heterozygote t-value divided by the trisomy t-value. Therefore, as the T-Score increases, the confidence of the trisomy call also increases. A T-Score greater than 5.0 is a confident trisomy call. A T-Score less than 0.6 indicates a confident heterozygous call.

Raw Data Plot vs. Corrected Data Plot



Other Considerations

Analysts trying to detect trisomy are challenged when aberrations caused by variation within an individual's own cells occur. Examples of individual variation include mixed samples, mosaicism, and triallelic homozygotes.

Maternal Cell Contamination (MCC)

Pregnant women over the age of 35 are screened for trisomy caused syndromes in the fetus because the risk of these syndromes increases as the mother ages. During the procedure whereby cells are collected for analysis, maternal cells can be mixed in with fetal cells. This phenomenon is called Maternal Cell Contamination (MCC). MCC is recognized when extra alleles appear and when examining inconclusive dosage ratios. When there are three alleles at a locus, the intensity of all three should be essentially equal. An imbalance is said to exist when the intensity ratio between the highest and lowest peak is greater than 60:40. The GeneMarker Trisomy tool identifies loci that contain imbalanced peaks. The user can define, within the settings parameters, the imbalance ratio that is significant for the data.

Mosaicism

Trisomy may not necessarily be present in all cells in an individual. It may be detected in just a specific tissue or within different cells in a tissue. When the presence of chromosomal abnormalities occurs differentially within an individual, it is called chromosomal mosaicism. In general, as we would expect, individuals who are mosaic for a chromosome change tend to have a less severe form of the syndrome present than full trisomy individuals. Critical examples of mosaicism are found in leukemia cases, specifically; chronic lymphocytic leukemia (CLL) which is a trisomy of chromosome 12 and acute myeloid leukemia (AML) prognosis which is a trisomy of chromosome 8. Detection of the smaller deviations from normal ratios caused by the presence of a population of trisomic cells in a single individual is possible with the GeneMarker Trisomy function.

Triallelic Homozygote

It is possible that an individual with three chromosomes could potentially have the same allele on all three chromosomes. In this instance, the electropherogram trace for this allele would theoretically depict a peak three times the height of a peak with just one allele. Since there is only one allele present in the marker and no other allele for intensity comparison, the analyst must use their own knowledge and experience to determine if the individual is a triallelic homozygote.

Reports and Printing

GeneMarker automates the analysis process and creates an easy-to-read report for aneuploidy determinations.

Report Table

Displays peak ratio and Trisomy Score for individual Markers in a sample. The *Electropherogram* frame will correspond to which ever peak cell is selected in the *Report Table*.

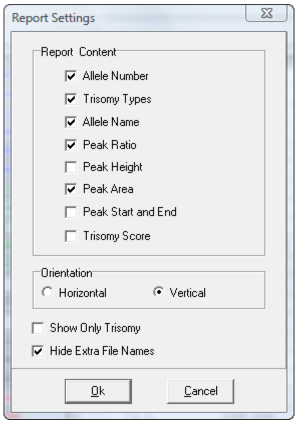
Report Settings
Launches the *Report Settings* dialog box.

Report Content
Choose peak and Marker values to display in the *Report Table*. The analysis type chosen in the *Trisomy Analysis Settings* box determines the *Report Content* initially chosen.
NOTE: When selected, *Peak Ratio* will correspond to the *Quantification* option chosen in the *Trisomy Analysis Settings* box (*Peak Height* or *Peak Area*).

Orientation
When *Horizontal* is chosen, Marker information will be displayed in columns along the top of the *Report Table*. When *Vertical* is chosen, Marker information will be displayed in rows.

Show Only Trisomy: When selected, only trisomy calls will be displayed in the *Report Table*, all other cells will be blank.

Hide Extra File Names: When selected, sample filenames will appear only once in the first row of the sample's information. Only available when *Vertical Orientation* is chosen.


Report Settings

Report Content
☑ Allele Number
☑ Trisomy Types
☑ Allele Name
☑ Peak Ratio
☐ Peak Height
☑ Peak Area
☐ Peak Start and End
☐ Trisomy Score

Orientation
○ Horizontal ● Vertical

☐ Show Only Trisomy
☑ Hide Extra File Names

Save Report
Choose to save the *Report Table* as an Excel file (.xls) or a tab-delimited Text file (.txt).

Trisomy Print Report Settings

Click the **Print** icon in the main toolbar to launch the *Trisomy Print Settings* box and preview, print or save the *Trisomy Print Report*.

NOTE: The analysis type chosen in the *Trisomy Analysis Settings* box determines the *Print Settings* initially chosen.

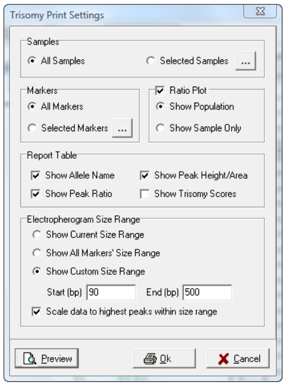
Samples
Choose *All Samples* or select specific samples to display in the report.

Markers
Choose *All Markers* or select specific Markers to display in the report.

Ratio Plot
When selected, a separate page, with the *Ratio Plot* for all Markers in a dye color, will be printed for each sample. Choose to *Show Population* to include all ratio data points in the dataset. Choose *Show Sample Only* to display only the sample's data points in the *Ratio Plot*.

Report Table
Select the information to display in the *Print Report's Report Table*.

Electropherogram Size Range
Show Current Size Range: Displays the Trisomy analysis window *Electropherogram* in its current zoom mode.
Show All Markers' Size Range: *Electropherograms* will be displayed so that all selected markers can be clearly viewed.
Show Custom Size Range: Expands the *Electropherogram* view to the specified range.
Scale Data to Highest Peaks Within the Size Range: Increases the peak heights of low peaks to approximately the same height as the highest peak in the dye color.


Trisomy Print Settings

Samples
● All Samples ○ Selected Samples

Markers
● All Markers
○ Selected Markers

☑ Ratio Plot
● Show Population
○ Show Sample Only

Report Table
☑ Show Allele Name ☑ Show Peak Height/Area
☑ Show Peak Ratio ☐ Show Trisomy Scores

Electropherogram Size Range
○ Show Current Size Range
○ Show All Markers' Size Range
● Show Custom Size Range
Start (bp) 90 End (bp) 500
☑ Scale data to highest peaks within size range

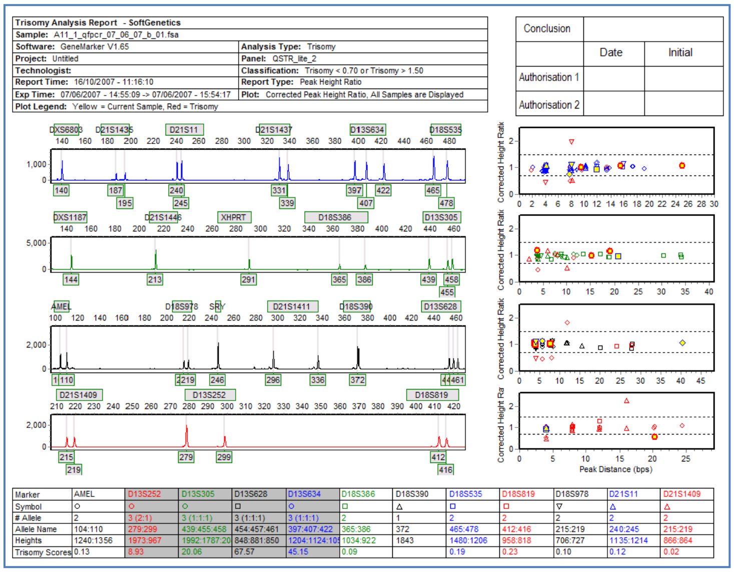
Trisomy Print Report

Below is a description of the *Trisomy Print Report* features. For an explanation of functions within the *Print Preview* window, see **Chapter 6 Reports and Printing.**

NOTE: The analysis type chosen in the *Trisomy Analysis Settings* box (*Classic*, *BPG* or *Aneuploidy*) determines the display of the resulting *Print Report*.

Classic Trisomy Print Report

When *Classic* is selected as the analysis type in the *Trisomy Analysis Settings* box, the *Print Report* example below is generated.



Trisomy Analysis Report - SoftGenetics	
Sample: A11_1_qfpcr_07_06_07_b_01.fsa	
Software: GeneMarker V1.65	**Analysis Type:** Trisomy
Project: Untitled	**Panel:** QSTR_lite_2
Technologist:	**Classification:** Trisomy < 0.70 or Trisomy > 1.50
Report Time: 16/10/2007 - 11:16:10	**Report Type:** Peak Height Ratio
Exp Time: 07/06/2007 - 14:55:09 -> 07/06/2007 - 15:54:17	**Plot:** Corrected Peak Height Ratio, All Samples are Displayed
Plot Legend: Yellow = Current Sample, Red = Trisomy	

Conclusion		
	Date	Initial
Authorisation 1		
Authorisation 2		

Marker	AMEL	D13S252	D13S305	D13S628	D13S634	D18S386	D18S390	D18S535	D18S819	D18S978	D21S11	D21S1409
Symbol	○	○	○	□	○	□	△	□	□	▽	△	△
# Allele	2	3 (2:1)	3 (1:1:1)	3 (1:1:1)	3 (1:1:1)	2	1	2	2	2	2	2
Allele Name	104:110	279:299	439:455:458	454:457:461	397:407:422	365:386	372	465:478	412:416	215:219	240:245	215:219
Heights	1240:1356	1973:967	1992:1787:20	848:881:850	1204:1124:10	1034:922	1843	1480:1206	958:818	706:727	1135:1214	866:864
Trisomy Scores	0.13	8.93	20.06	67.57	45.15	0.09		0.19	0.23	0.10	0.12	0.02

DXS6803
D21S1435
D21S11
D21S1437
D13S634
D18S535
DXS1187
D21S1446
XHPRT
D18S386
D13S305
AMEL
D18S978
SRY
D21S1411
D18S390
D13S628
D21S1409
D13S252
D18S819
Corrected Height Ratio
Peak Distance (bps)

Report Header

Contains information about the analysis, project, sample and parameters.

Signature Box

Date and initial space for report reviewers.

Electropherogram

Similar to the Trisomy analysis window, displays all dye colors of the sample trace.

Corrected Ratio Plot

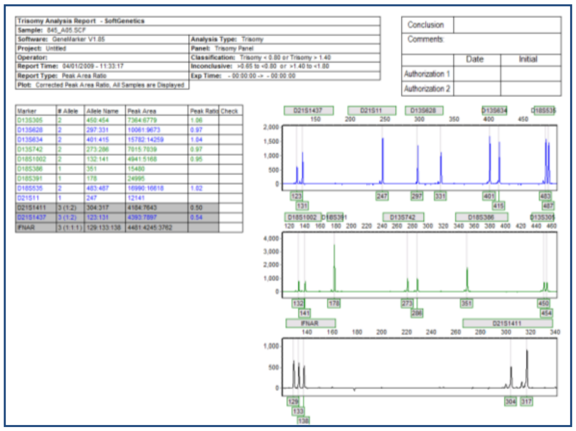
Contains the entire dataset's plot points for all Markers in the dye color. Symbol shapes represent different Markers and can be deciphered from the *Symbol* row in the *Report Table*. Yellow filled symbols represent the current sample's data points. Red outlined symbols represent trisomy calls.

Report Table

Displays selected peak and Marker values for the current sample. Trisomy calls are highlighted grey.

Best Practices Guideline (BPG) and Aneuploidy Print Reports

When *BPG or Aneuploidy* is selected as the analysis type in the *Trisomy Analysis Settings* box, the *Print Report* example below is generated. Grayed files indicate that the ratio is consistent with trisomy and '?' indicates that the ratio is within the inconclusive range.



Trisomy Analysis Report - SoftGenetics	
Sample: 845_A05.SCF	
Software: GeneMarker V1.85	**Analysis Type:** Trisomy
Project: Untitled	**Panel:** Trisomy Panel
Operator:	**Classification:** Trisomy < 0.80 or Trisomy > 1.40
Report Time: 04/01/2009 - 11:33:17	**Inconclusive:** >0.65 to <0.80 or >1.40 to <1.80
Report Type: Peak Area Ratio	**Exp Time:** - 00:00:00 -> - 00:00:00
Plot: Corrected Peak Area Ratio, All Samples are Displayed	

Conclusion		
Comments		
	Date	Initial
Authorization 1		
Authorization 2		

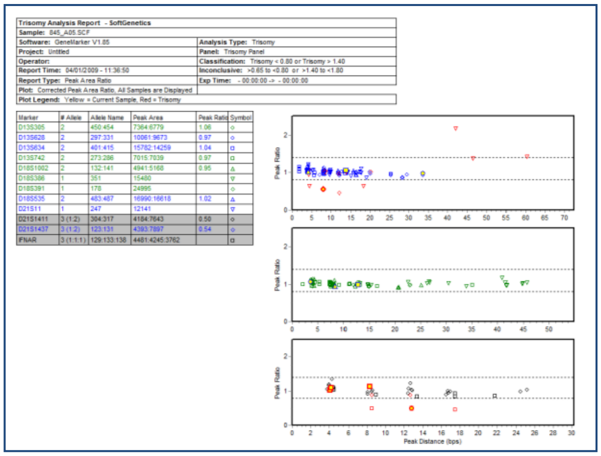
Marker	# Allele	Allele Name	Peak Area	Peak Ratio	Check
D13S305	2	450:454	7364:6779	1.06	
D13S628	2	297:331	10061:9673	0.97	
D13S634	2	401:415	15782:14259	1.04	
D13S742	2	273:286	7015:7039	0.97	
D18S1002	2	132:141	4941:5168	0.95	
D18S386	1	351	15480		
D18S391	1	178	24995		
D18S535	2	483:487	16990:16618	1.02	
D21S11	1	247	12141		
D21S1411	3 (1:2)	304:317	4184:7643	0.50	
D21S1437	3 (1:2)	123:131	4393:7897	0.54	
IFNAR	3 (1:1:1)	129:133:138	4481:4245:3762		

Report Header
Contains information about the analysis, project, sample and parameters.

Signature Box
Date and initial space for report reviewers.

Electropherogram
Similar to the Trisomy analysis window, displays all dye colors of the sample trace.

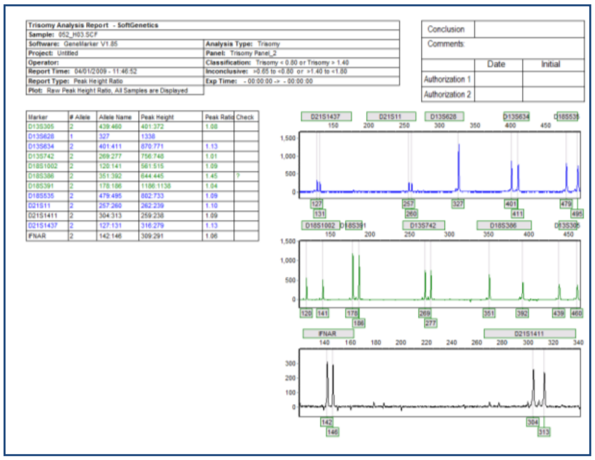
Report Table
Displays selected peak and Marker values for the current sample. Trisomy calls are highlighted grey. An additional *Check* column is provided for indication of inconclusive range for trisomy and reviewer initials.



Trisomy Analysis Report - SoftGenetics	
Sample: 845_A05.SCF	
Software: GeneMarker V1.85	**Analysis Type:** Trisomy
Project: Untitled	**Panel:** Trisomy Panel
Operator:	**Classification:** Trisomy < 0.80 or Trisomy > 1.40
Report Time: 04/01/2009 - 11:36:50	**Inconclusive:** >0.65 to <0.80 or >1.40 to <1.80
Report Type: Peak Area Ratio	**Exp Time:** - 00:00:00 -> - 00:00:00
Plot: Corrected Peak Area Ratio, All Samples are Displayed	
Plot Legend: Yellow = Current Sample, Red = Trisomy	

Marker	# Allele	Allele Name	Peak Area	Peak Ratio	Symbol
D13S305	2	450:454	7364:6779	1.06	◇
D13S628	2	297:331	10061:9673	0.97	○
D13S634	2	401:415	15782:14259	1.04	□
D13S742	2	273:286	7015:7039	0.97	□
D18S1002	2	132:141	4941:5168	0.95	△
D18S386	1	351	15480		▽
D18S391	1	178	24995		◇
D18S535	2	483:487	16990:16618	1.02	△
D21S11	1	247	12141		▽
D21S1411	3 (1:2)	304:317	4184:7643	0.50	○
D21S1437	3 (1:2)	123:131	4393:7897	0.54	◇
IFNAR	3 (1:1:1)	129:133:138	4481:4245:3762		□

Corrected Ratio Plot
Contains the entire dataset's plot points for all Markers in the dye color. Symbol shapes represent different Markers and can be deciphered from the *Symbol* row in the *Report Table*. Yellow filled symbols represent the current sample's data points. Red outlined symbols represent trisomy calls.



Trisomy Analysis Report - SoftGenetics	
Sample: 052_H03.SCF	
Software: GeneMarker V1.85	**Analysis Type:** Trisomy
Project: Untitled	**Panel:** Trisomy Panel_2
Operator:	**Classification:** Trisomy < 0.80 or Trisomy > 1.40
Report Time: 04/01/2009 - 11:46:52	**Inconclusive:** >0.65 to <0.80 or >1.40 to <1.80
Report Type: Peak Height Ratio	**Exp Time:** - 00:00:00 -> - 00:00:00
Plot: Raw Peak Height Ratio, All Samples are Displayed	

Conclusion		
Comments		
	Date	Initial
Authorization 1		
Authorization 2		

Marker	# Allele	Allele Name	Peak Height	Peak Ratio	Check
D13S305	2	439:460	401:372	1.08	
D13S628	1	327	1338		
D13S634	2	401:411	870:771	1.13	
D13S742	2	269:277	756:748	1.01	
D18S1002	2	120:141	561:515	1.09	
D18S386	2	351:392	644:445	1.45	?
D18S391	2	178:186	1186:1138	1.04	
D18S535	2	479:495	802:733	1.09	
D21S11	2	257:260	262:239	1.10	
D21S1411	2	304:313	259:238	1.09	
D21S1437	2	127:131	316:279	1.13	
IFNAR	2	142:146	309:291	1.06	

NOTE: The *Corrected Ratio Plot* appears on a second page for each sample only when *Ratio Plot* is selected in the *Trisomy Print Report Settings* box.

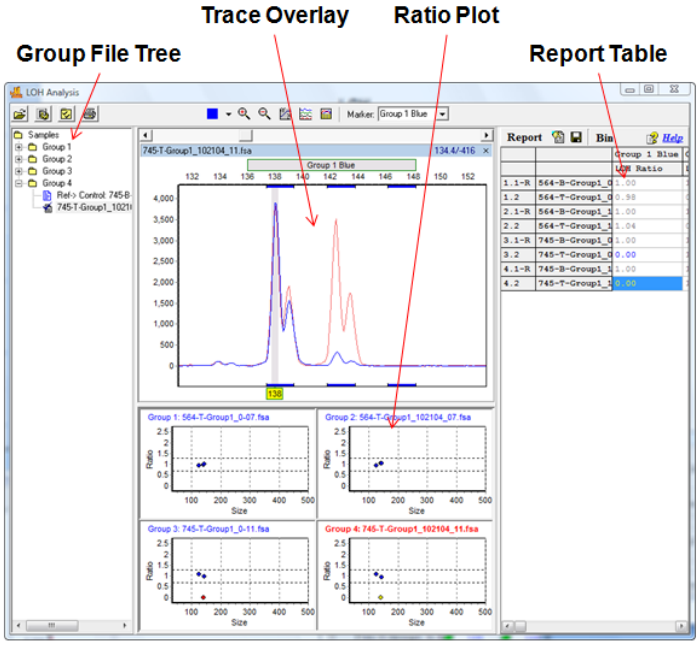
Loss of Heterozygosity (LOH)

Loss of Heterozygosity (LOH) occurs when a somatic cell contains only one copy of an allele due to non-disjunction during mitosis, segregation during recombination, or deletion of a chromosome segment. LOH becomes critical when the remaining allele contains a point mutation that renders the gene inactive. This is a common occurrence in cancers where a tumor suppressor gene is affected. Tumor suppressor genes code for proteins that regulate the cell's life cycle. Thus, they are critical in preventing tumor formation.

Overview

GeneMarker fragment analysis software has been developed to aid researchers and clinicians in the detection of LOH within cancer cells. Using a patented allele calling algorithm, GeneMarker uses the germ line reference trace to compare and detect LOH in patient samples.

LOH Analysis


Trace Overlay
Ratio Plot
Group File Tree
Report Table
LOH Analysis
Marker: Group 1 Blue
Samples
Group 1
Group 2
Group 3
Group 4
Ref-> Control: 745-B-
745-T-Group1_1021
745-T-Group1_102104_11.fsa
134.4/-416
Group 1 Blue
132
134
136
138
140
142
144
146
148
150
152
4,000
3,500
3,000
2,500
2,000
1,500
1,000
500
0
138
Group 1: 564-T-Group1_0-07.fsa
Group 2: 564-T-Group1_102104_07.fsa
Group 3: 745-T-Group1_0-11.fsa
Group 4: 745-T-Group1_102104_11.fsa
Ratio
Size
Report
Bin
Help

		Group 1 Blue
		LOH Ratio
1.1-R	564-B-Group1_0	1.00
1.2	564-T-Group1_0	0.98
2.1-R	564-B-Group1_1	1.00
2.2	564-T-Group1_1	1.04
3.1-R	745-B-Group1_0	1.00
3.2	745-T-Group1_0	0.00
4.1-R	745-B-Group1_1	1.00
4.2	745-T-Group1_1	0.00

Group File Tree

Samples in the *Group File Tree* appear within Group folders according to the information provided in the *File Name Group Text* file uploaded in the *LOH Analysis Settings* box. A sample is marked as the Reference because it contains a *Control Identifier* as set in the *File Name Group* tool. **See Chapter 8 Additional Tools**. Reference/Control samples will appear in the first column of the *File Name Group Text* file.

Expand folders in the *Group File Tree* to view the Reference sample and Experimental sample in the group. Double-click the Reference sample to view just the Reference sample electropherogram. Double-click the Experimental sample to view the Experimental sample electropherogram overlaid on the Reference sample electropherogram and associated *Gain/Loss Histogram*. Use the **Up/Down Arrow** keys to navigate through samples. Hit the **Enter** key to open Group folders.

Right-click an Experimental sample in the *Group File Tree* and select *Set as Reference* to mark the sample as the Group's Reference sample.

Trace Overlay

The *Trace Overlay* displays the selected Experimental sample's trace superimposed over the selected Group's Reference trace. The Experimental sample trace line color will appear in the Marker's associated dye color. The Reference trace line color is displayed in light red. The Reference trace is, by default, drawn directly behind the Experimental sample trace. Click the **LOH Display Settings** icon to adjust the Reference trace offset as compared to the Experimental sample trace. Additionally, the RFU intensity or peak height of the Reference trace is normalized to the Experimental sample traces in the Group. To disable Reference trace normalization, click the **LOH Display Settings** icon and deselect *Peak Normalization*. The uncorrected Reference trace peak heights will be displayed in the *Trace Overlay*.

The allele editing options in the *Trace Overlay* frame are similar to those in the *Main Analysis* window *Electropherogram*. **See Chapter 3 Main Analysis Overview**.

Ratio Plot

The *Ratio Plot* depicts graphically peak ratio versus basepair position. The peak ratio value is calculated by first determining the height or area ratio of peaks within a Marker in the Experimental and Reference sample separately. The Experimental and Reference ratios are then compared and a final peak ratio is determined and plotted in the *Ratio Plot*. Select *Peak Height* or *Peak Area* in the *Quantification by* section of the *LOH Analysis Settings* box. Ratio thresholds are set in the LOH Analysis Settings box. If a plot point occurs outside of the ratio thresholds then the point will appear red. Double-click plot points to view the trace in the *Trace Overlay* frame. The number of Ratio Plots displayed at one time can be adjusted in the *LOH Display Settings* box.

Report Table

The *Report Table* lists all samples in a column on the left. Reference samples are marked with an "R". Click the **Report Settings** icon to select display options. Double-click values in the *Report Table* to link to the associated *Trace Overlay* position. Click the **Save Report** icon to save the *Report Table* as an Excel or tab-delimited Text file.

Procedure

1. Import LOH raw data files and filter with Run Wizard **Fragment (Animal) Analysis** settings. **See Chapter 2 General Procedure.**
2. Create a Panel for the data with the *Panel Editor* tool. **See Chapter 5 Panel Editor.**
3. Apply the Panel
4. Select *Applications → LOH Analysis*
5. The *LOH Analysis Settings* box appears
6. Upload a tab-delimited text file that identifies Reference samples (first column) and Experimental samples (second column) to the *Group File* field

NOTE: Use the *File Name Group Tool* to create a group file. **See Chapter 8 Additional Tools**.

7. Adjust *LOH Analysis Settings* as desired
8. Click **OK**
9. The *LOH Analysis* window appears
10. Click the **Print** icon to print the *LOH Clinical Report*

Icons and Functions

Load Group Information

Choose a tab-delimited Text (.txt) file which contains information on how to group individual sample files. For example, pair Patient A's tumor sample to Patient A's normal sample by placing the normal sample filename in the first column and the tumor sample in the second column. **See Chapter 8 Additional Tools.**

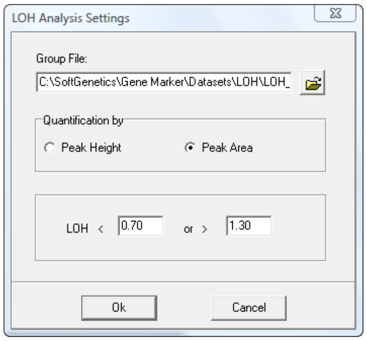
Analysis Settings

Opens the *LOH Analysis Settings* box.

Group File

Click *Open File* icon to upload a .txt file that groups samples by filename.

Quantification



Group File: C:\SoftGenetics\Gene Marker\Datasets\LOH\LOH_

Quantification by
○ Peak Height ● Peak Area

LOH < 0.70 or > 1.30

Peak Height: When selected, LOH peak ratios will be calculated based on the RFU intensity values of a peak.
Peak Area: When selected, LOH peak ratios will be calculated based on the area under the curve of the peak.

LOH
The ratio between reference and sample, based on height or area, calculated for each individual peak to determine presence or absence of the peak.

Layout Settings
Launches the *LOH Display Settings* box.

Ctrl/Sample Shift
Adjust the amount of offset between the Reference trace and the Experimental sample trace in the *Trace Overlay* electropherogram.

Peak Normalization
When selected, Reference sample peaks will be normalized based on peak height or area to the Experimental sample peaks in the Group.

Max Row/Column
Select the number of *Ratio Plots* to display at one time in the *LOH Analysis* window.

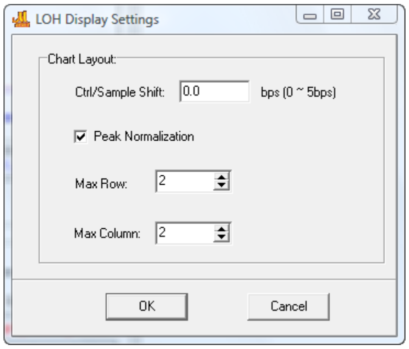
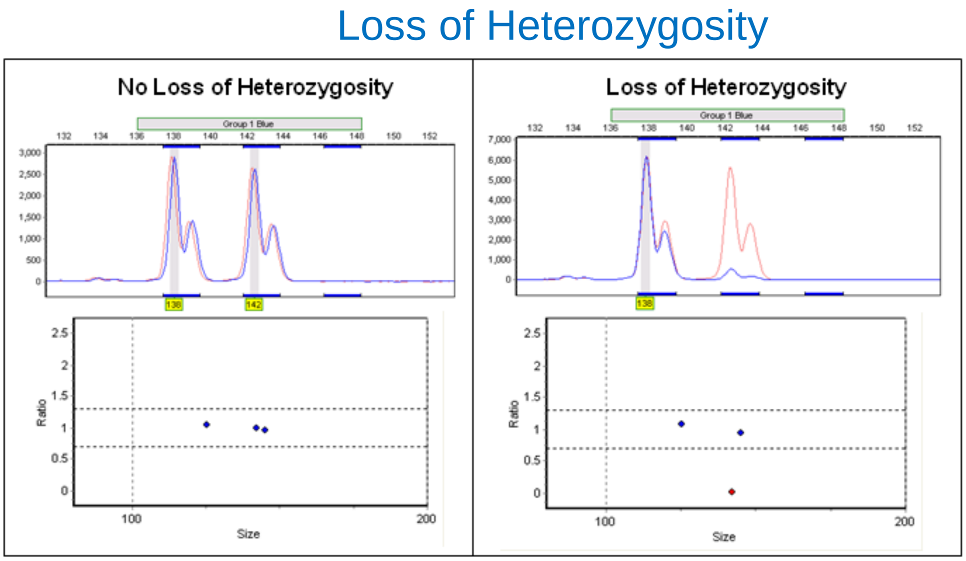


Chart Layout:
Ctrl/Sample Shift: 0.0 bps (0 ~ 5bps)
☑ Peak Normalization
Max Row: 2
Max Column: 2

Print
Launches the *LOH Print Settings* box. See *Reports and Printing* section below for more information.

What to Expect

The concept for determining LOH is the comparison of a suspect tissue sample to a normal tissue sample with all expected alleles. In GeneMarker, a patient trace is compared to a reference trace. Samples with loss of heterozygosity are immediately apparent in two displays – the electropherogram and the ratio plot. In the electropherogram, the reference trace is in light red behind the patient trace. When a peak is absent in the patient trace, only the reference trace peak remains. In the ratio plot, the absence of a patient trace peak is indicated by a red dot that falls outside the user-defined bounds of the LOH ratio (indicated by dashed lines). Blue dots within the LOH ratio bounds indicate a patient trace with the expected number of peaks.



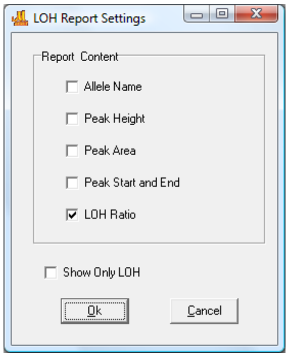
In addition to the ratio plot, a clinician can use the LOH score parameter in the report table to qualify a given marker. LOH score is calculated using the peak intensity or area ratio between normal and tumor samples. For complementary peaks in both reference and sample trace the score will equal 1.0. In the case of complete loss of heterozygosity at a certain position, the LOH score will be 0.0.

Reports and Printing

Loss of heterozygosity detection is a key component in separating cancerous from non-cancerous tissue. The ability to do this type of analysis quickly and accurately is an extreme advantage to clinicians determined to fight the disease. In addition to GeneMarker's LOH analysis tool, an easy-to-read report can be printed for clinical review. The LOH Clinical Report includes patient/reference traces, ratio plots and a table of peak statistics.

Report Table

Displays peak loss information for individual samples compared to a reference. Information for samples with suspected LOH will appear in blue font, all others in gray. The Overlay Trace window will correspond to which ever peak cell is selected in the Report Table.



LOH Report Settings

Report Content
☐ Allele Name
☐ Peak Height
☐ Peak Area
☐ Peak Start and End
☑ LOH Ratio

☐ Show Only LOH

Report Settings

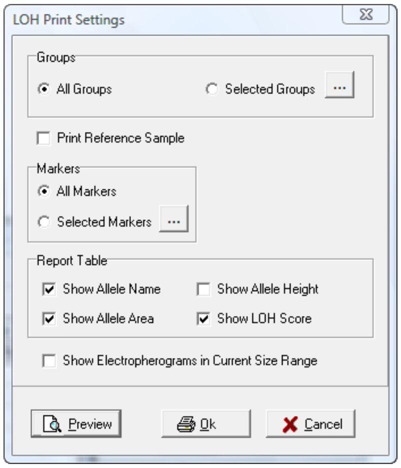
Report Content
Select to display Allele Name, Peak Height, Peak Area, Peak Start and End, and/or LOH Ratio. LOH Ratio is chosen by default.

Show Only LOH
When selected, only the report content information for suspected LOH samples will be displayed.

Save Report
Choose to save the Report Table as an Excel file (.xls) or a tab-delimited Text file (.txt).

LOH Clinical Report Settings

Click the **Print** icon in the main tool bar to launch the *LOH Print Settings* box and preview, print or save the *LOH Analysis Report*.



LOH Print Settings

Groups
● All Groups ○ Selected Groups

☐ Print Reference Sample

Markers
● All Markers
○ Selected Markers

Report Table
☑ Show Allele Name ☐ Show Allele Height
☑ Show Allele Area ☑ Show LOH Score

☐ Show Electropherograms in Current Size Range

Groups
Choose *All Groups* or select specific groups to display in the report.

Print Reference Sample
When selected, a separate page with just the reference sample will be printed for each group.

Markers
Choose *All Markers* or select specific markers to display in the report.

Report Table
Choose the contents of the report table.

Show Electropherograms in Current Size Range
When selected, will display *Overlay Trace* electropherograms in the same zoom mode as the main *LOH Analysis* window. When deselected, the electropherograms will be displayed so that all selected Markers can be clearly viewed.

LOH Clinical Report

Below is a description of the *LOH Clinical Report* features. For an explanation of functions within the *Print Preview* window, see **Chapter 6 Reports and Printing.**

Report Header
Contains information about the analysis, project, sample and parameters.

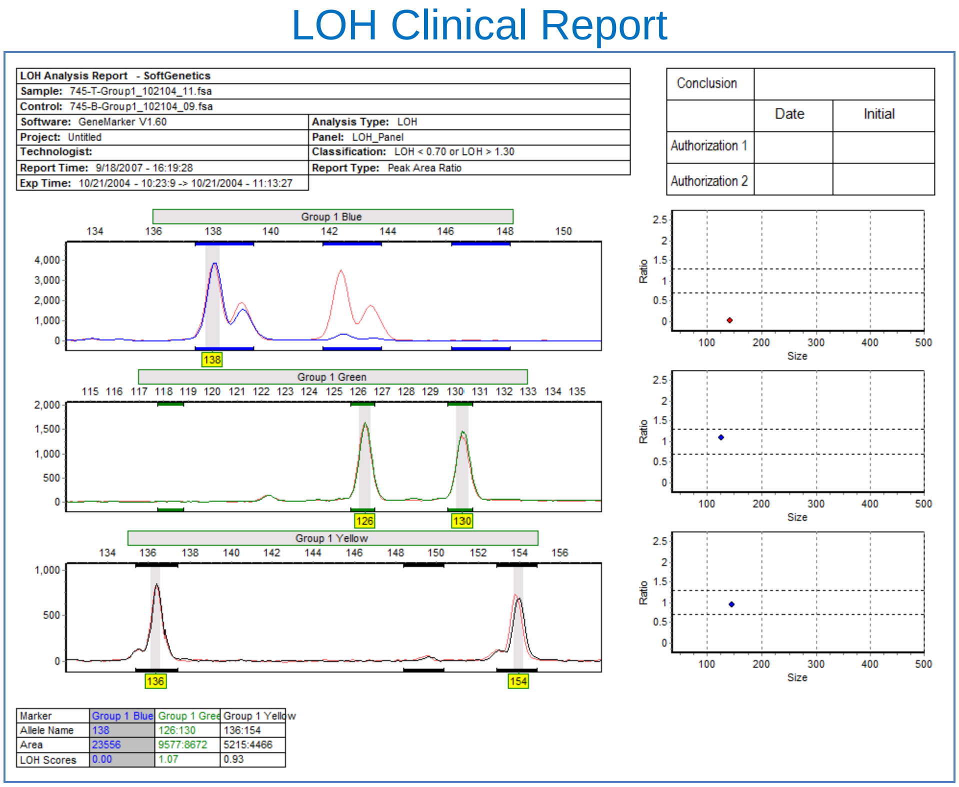
Signature Box

Date and initial space for report reviewers.

Overlay Trace Electropherogram
Similar to the *LOH Analysis* window, displays the Reference trace in light red behind the Experimental trace.

Ratio Plot
Also similar to the *LOH Analysis* window, displays a ratio plot of reference versus sample based on peak height or area and can be adjusted in the *LOH Analysis Settings* box.

Report Table
Identifies the marker and statistical information chosen in the *LOH Print Settings* box.



LOH Clinical Report

LOH Analysis Report - **SoftGenetics**
Sample: 745-T-Group1_102104_11.fsa
Control: 745-B-Group1_102104_09.fsa
Software: GeneMarker V1.60
Analysis Type: LOH
Project: Untitled
Panel: LOH_Panel
Technologist:
Classification: LOH < 0.70 or LOH > 1.30
Report Time: 9/18/2007 - 16:19:28
Report Type: Peak Area Ratio
Exp Time: 10/21/2004 - 10:23:9 -> 10/21/2004 - 11:13:27

Conclusion		
	Date	Initial
Authorization 1		
Authorization 2		

Group 1 Blue
134
136
138
140
142
144
146
148
150
4,000
3,000
2,000
1,000
0
138
Group 1 Green
115 116 117 118 119 120 121 122 123 124 125 126 127 128 129 130 131 132 133 134 135
2,000
1,500
1,000
500
0
126
130
Group 1 Yellow
134
136
138
140
142
144
146
148
150
152
154
156
1,000
500
0
136
154
Ratio
2.5
2
1.5
1
0.5
0
100
200
300
400
500
Size

Marker	Group 1 Blue	Group 1 Gree	Group 1 Yellow
Allele Name	138	126:130	136:154
Area	23556	9577:8672	5215:4466
LOH Scores	0.00	1.07	0.93

TILLING Analysis

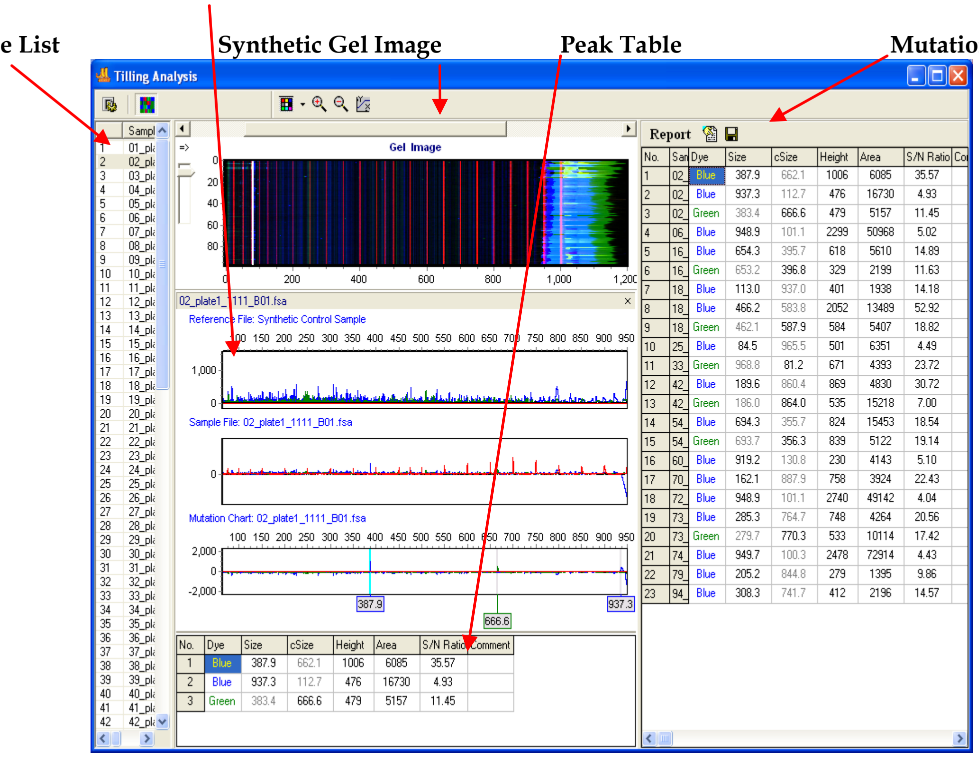
The techniques of Targeted Induced Local Lesions In Genomes, (TILLING®) and EcoTILLING have been widely used since 2000 to detect Single Nucleotide Polymorphisms (SNPs). During TILLING the test samples may be experimentally mutagenized (ethylmethanesulfonate, radiation, etc.) or from natural populations or derived from tumors or diseased tissues. Briefly, the genes of interest are identified with gene-specific primers and PCR amplified. The amplicon's primers are labeled with two fluorescent dyes – the forward is often labeled with FAM-blue and the reverse primer is often labeled with HEX-green. The samples are mixed so heteroduplexes can be formed. The hybridized fragments are cleaved at the heteroduplex site by CEL I or Surveyor™ Nuclease, generating multiple pairs of fragments of complementary length and dye color. The denatured samples can be run through gel electrophoresis or mixed with an internal size standard and run through capillary electrophoresis. SNPs will yield two peaks of different color and the sum of the sizes will equal the amplicon length.

Overview

GeneMarker TILLING analysis software has been optimized for detecting SNPs generated by the TILLING technique. By using the internal size standard to align each capillary and generating a reference trace from all of the samples in the run, the reference can be subtracted from each individual sample trace yielding a plot highlighting the SNPs. Additionally, a table shows what the expected size of the complementary fragment should be to determine if each peak is a true variation.

The TILLING analysis window displays a synthetic gel image, list of samples, sample electropherogram and a report table. The information contained in the report is interlinked to the sample traces. Double-clicking on the cell in the report highlights the cell, corresponding sample ID in the sample list and locus in the electropherogram.

TILLING Analysis


Reference, Sample, Subtracted sample/mutation Traces
Sample List
Synthetic Gel Image
Peak Table
Mutation Report
Tilling Analysis
Sampl
Gel Image
0
20
40
60
80
200
400
600
800
1,000
1,200
02_plate1_1111_B01.fsa
Reference File: Synthetic Control Sample
Sample File: 02_plate1_1111_B01.fsa
Mutation Chart: 02_plate1_1111_B01.fsa
100 150 200 250 300 350 400 450 500 550 600 650 700 750 800 850 900 950
1,000
2,000
-2,000
387.9
666.6
937.3
Report

No.	Dye	Size	cSize	Height	Area	S/N Ratio	Comment
1	Blue	387.9	662.1	1006	6085	35.57	
2	Blue	937.3	112.7	476	16730	4.93	
3	Green	383.4	666.6	479	5157	11.45	

No.	San	Dye	Size	cSize	Height	Area	S/N Ratio	Co
1	02_	Blue	387.9	662.1	1006	6085	35.57	
2	02_	Blue	937.3	112.7	476	16730	4.93	
3	02_	Green	383.4	666.6	479	5157	11.45	
4	06_	Blue	948.9	101.1	2299	50968	5.02	
5	16_	Blue	654.3	395.7	618	5610	14.89	
6	16_	Green	653.2	396.8	329	2199	11.63	
7	18_	Blue	113.0	937.0	401	1938	14.18	
8	18_	Blue	466.2	583.8	2052	13489	52.92	
9	18_	Green	462.1	587.9	584	5407	18.82	
10	25_	Blue	84.5	965.5	501	6351	4.49	
11	33_	Green	968.8	81.2	671	4393	23.72	
12	42_	Blue	189.6	860.4	869	4830	30.72	
13	42_	Green	186.0	864.0	535	15218	7.00	
14	54_	Blue	694.3	355.7	824	15453	18.54	
15	54_	Green	693.7	356.3	839	5122	19.14	
16	60_	Blue	919.2	130.8	230	4143	5.10	
17	70_	Blue	162.1	887.9	758	3924	22.43	
18	72_	Blue	948.9	101.1	2740	49142	4.04	
19	73_	Blue	285.3	764.7	748	4264	20.56	
20	73_	Green	279.7	770.3	533	10114	17.42	
21	74_	Blue	949.7	100.3	2478	72914	4.43	
22	79_	Blue	205.2	844.8	279	1395	9.86	
23	94_	Blue	308.3	741.7	412	2196	14.57	

Sample List

The Sample List displays all current samples in the project. Single left-click a sample name or use the Up/Down Arrow keys to scroll through the list. Right click on a sample name for options on selecting/deselecting, sample information, sorting, disabling samples or editing comments.

Reference, Sample, Subtracted sample/mutation Traces

The reference file will be subtracted from each individual sample trace (center trace), yielding a plot (Mutation Chart) highlighting the SNPs.

Peak Table

The called peak size will be reported in bold type and complementary size (cSize) will be reported in grayed-out font.

Mutation Report

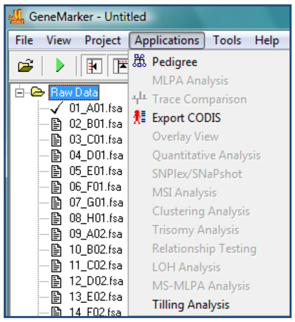
The information contained in the report is interlinked to the sample traces. Double-clicking on the cell in the report highlights the cell, corresponding sample ID in the sample list and locus in the electropherogram. Click the Report Settings icon to select display options. Click the Save Report icon to save the Report Table as an Excel or tab-delimited Text file

Procedure

1. Launch GeneMarker software. Import raw data files See Chapter 2 General Procedure
2. Select Tilling Analysis from Applications menu
3. Select Size Standard and Standard Color, Data Process Parameters and Data Range Parameters from pull-down menus
4. Click OK to process data
5. The report table can be saved in TXT file format

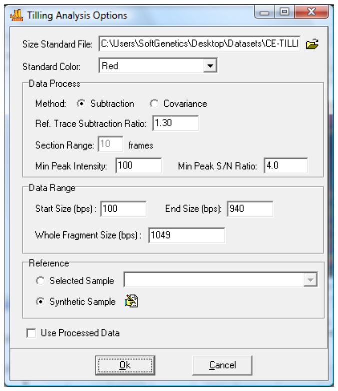
Icons and Functions

Open files icon and navigate to the file of interest click Add, navigate to directory containing data of interest, select data files (up to 1000 lanes) → click Open and OK.


GeneMarker - Untitled
File
View
Project
Applications
Tools
Help
Raw Data
01_A01.fsa
02_B01.fsa
03_C01.fsa
04_D01.fsa
05_E01.fsa
06_F01.fsa
07_G01.fsa
08_H01.fsa
09_A02.fsa
10_B02.fsa
11_C02.fsa
12_D02.fsa
13_E02.fsa
14_F02.fsa
Pedigree
MLPA Analysis
Trace Comparison
Export CODIS
Overlay View
Quantitative Analysis
SNPlex/SNaPshot
MSI Analysis
Clustering Analysis
Trisomy Analysis
Relationship Testing
LOH Analysis
MS-MLPA Analysis
Tilling Analysis

Select Tilling Analysis from the Applications menu to activate the Tilling Analysis Options dialog box

Select Size Standard
[C:\ProgramFiles\SoftGenetics\GeneMarker\1.90(Val)\SizeStd\SGSize_Rox1000.xml—if default installation location was accepted] and dye color. If a size standard used is other than one that is listed, the user can quickly construct one using the Size Template Editor (See Chapter 4).
Enter **Data Process options -** For a Ref. Trace Subtraction Ratio, value of 1.5: the software will subtract 1.3 times the reference trace from the sample trace**.**
Signal-to-Noise ratio—the software compares the called peak to other peaks in the neighborhood
Data Range: Enter the start and end size values for viewing the data; Enter the expected size of the undigested fragment.


Tilling Analysis Options
Ok
Cancel

Size Standard File: C:\Users\SoftGenetics\Desktop\Datasets\CE-TILLI
Standard Color: Red
Data Process
Method: ● Subtraction ○ Covariance
Ref. Trace Subtraction Ratio: 1.30
Section Range: 10 frames
Min Peak Intensity: 100
Min Peak S/N Ratio: 4.0
Data Range
Start Size (bps) : 100
End Size (bps): 940
Whole Fragment Size (bps) : 1049
Reference
○ Selected Sample
● Synthetic Sample
☐ Use Processed Data

TILLING Analysis Setting - Launches the TILLING *Analysis Settings* box from an open TILLING ANALYSIS WINDOW

Tilling Analysis window: Toolbar icons

Show/Hide Gel Image displays selected samples as a synthetic gel image. Bin ranges in the *Gel Image* mode appear as white vertical lines and can be manipulated by holding down **SHIFT** and dragging the white lines left or right.

Show Color Allows the user to select all colors to view, hide all colors, or choose a single dye layer. Choose a single dye by single left mouse clicking on the icon.

Zoom In Use the icon to zoom in on the image, or hold down the left mouse button and draw a box, from the top left corner to bottom right corner, around the area you wish to zoom in on.

Zoom Out Use the icon to zoom out on the image, or hold down the left mouse button and draw a box, from the bottom right corner to top left corner.

Set Axis The default setting automatically sets the Y-axis according to the maximum peak intensity of the samples. Two other options are available: auto fit the Y-axis using peak intensities of the alleles, or the user can select the ranges for the X- and Y-axis.

Editing peaks: if a peak is called that you determine should be deleted (S/N ratio close to cut-off value or no complementary fragment observed) right-click on peak in mutation chart trace and select delete from the pop-up menu. The deleted peak can be undeleted or an uncalled peak can be added using the same pop-up menu.

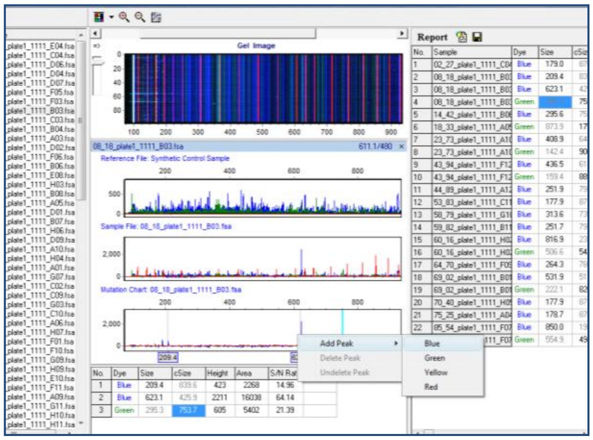

Gel Image
0
20
40
60
80
100
200
300
400
500
600
700
800
900
08_18_plate1_1111_B03.fsa
611.1/480
Reference File: Synthetic Control Sample
Sample File: 08_18_plate1_1111_B03.fsa
Mutation Chart: 08_18_plate1_1111_B03.fsa
500
2,000
209.4
Add Peak
Delete Peak
Undelete Peak
Blue
Green
Yellow
Red
Report

plate1_1111_E04.fsa
plate1_1111_C04.fsa
plate1_1111_D06.fsa
plate1_1111_D04.fsa
plate1_1111_D07.fsa
plate1_1111_F05.fsa
plate1_1111_F03.fsa
plate1_1111_B03.fsa
plate1_1111_C03.fsa
plate1_1111_B04.fsa
plate1_1111_A03.fsa
plate1_1111_D02.fsa
plate1_1111_F06.fsa
plate1_1111_B06.fsa
plate1_1111_E08.fsa
plate1_1111_H03.fsa
plate1_1111_B08.fsa
plate1_1111_A05.fsa
plate1_1111_D01.fsa
plate1_1111_B07.fsa
plate1_1111_H06.fsa
plate1_1111_D09.fsa
plate1_1111_A10.fsa
plate1_1111_H04.fsa
plate1_1111_A01.fsa
plate1_1111_G07.fsa
plate1_1111_C02.fsa
plate1_1111_C09.fsa
plate1_1111_G03.fsa
plate1_1111_C10.fsa
plate1_1111_A06.fsa
plate1_1111_H07.fsa
plate1_1111_F01.fsa
plate1_1111_F10.fsa
plate1_1111_G09.fsa
plate1_1111_H09.fsa
plate1_1111_E10.fsa
plate1_1111_F11.fsa
plate1_1111_A09.fsa
plate1_1111_G11.fsa
plate1_1111_H10.fsa
plate1_1111_H11.fsa

No.	Dye	Size	cSize	Height	Area	S/N Rat
1	Blue	209.4	839.6	423	2268	14.96
2	Blue	623.1	425.9	2211	16038	64.14
3	Green	295.3	753.7	605	5402	21.39

No.	Sample	Dye	Size	cSiz
1	02_27_plate1_1111_C04	Blue	179.0	67
2	08_18_plate1_1111_B03	Blue	209.4	83
3	08_18_plate1_1111_B03	Blue	623.1	42
4	08_18_plate1_1111_B03	Green	[illegible]	75
5	14_42_plate1_1111_B06	Blue	295.6	75
6	18_33_plate1_1111_A05	Green	873.9	17
7	23_73_plate1_1111_A10	Blue	408.9	64
8	23_73_plate1_1111_A10	Green	142.4	90
9	43_94_plate1_1111_F12	Blue	436.5	61
10	43_94_plate1_1111_F12	Green	159.4	88
11	44_89_plate1_1111_A12	Blue	251.9	79
12	53_83_plate1_1111_C11	Blue	177.9	87
13	58_79_plate1_1111_G10	Blue	313.6	73
14	59_82_plate1_1111_B11	Blue	251.7	79
15	60_16_plate1_1111_H02	Blue	816.9	23
16	60_16_plate1_1111_H02	Green	506.6	54
17	64_70_plate1_1111_F09	Blue	264.3	78
18	69_02_plate1_1111_B01	Blue	531.9	51
19	69_02_plate1_1111_B01	Green	222.1	82
20	70_40_plate1_1111_H05	Blue	177.9	87
21	75_25_plate1_1111_A04	Blue	178.7	87
22	85_54_plate1_1111_F07	Blue	850.0	19
	111_F07	Green	554.9	49

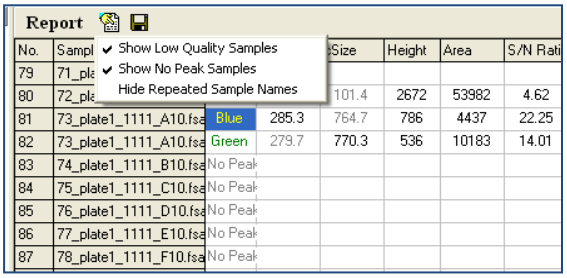
What to Expect

Increased sensitivity is a big advantage to using GeneMarker. EcoTILLING samples, which may contain one mutated portion out of 12 pooled samples, may yield very low levels of the cleaved fragments. We are able to detect variations in EcoTILLING samples where the variant may contribute to only 4% of sample. Some researchers have found 30% more DNA variations by using this tool. In some cases, the intensity of the SNP may fall below the level of noise and making visual inspection impossible – these variants can now be found when the reference noise is removed from the sample. Because the sum of the sizes of each color should equal the whole amplicon size, we are able to detect the cleavage sites in EcoTILLING sample using GeneMarker's Tilling Application module.

False Positives, often caused by chemistry error, random cleavage and instrument spikes, can easily be identified and removed. Spikes often show multiple colors at the same location. The peaks caused by these random processes seldom occur in a complementary size.

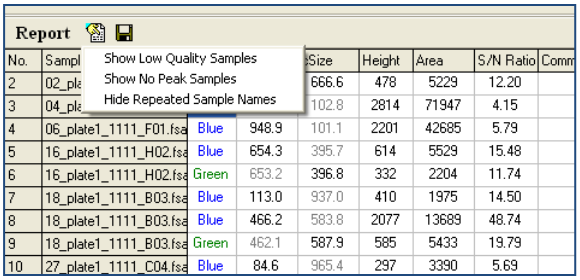
Reports

Save Report - Choose to save the *Report Table* as an Excel file (.xls) or a tab-delimited Text file (.txt).


Report

No.	Sampl			Size	Height	Area	S/N Rati
79	71_pla						
80	72_pla			101.4	2672	53982	4.62
81	73_plate1_1111_A10.fsa	Blue	285.3	764.7	786	4437	22.25
82	73_plate1_1111_A10.fsa	Green	279.7	770.3	536	10183	14.01
83	74_plate1_1111_B10.fsa	No Peak					
84	75_plate1_1111_C10.fsa	No Peak					
85	76_plate1_1111_D10.fsa	No Peak					
86	77_plate1_1111_E10.fsa	No Peak					
87	78_plate1_1111_F10.fsa	No Peak					

Report Options - The report table can be configured to report all results, positive and negative or to display samples containing only positive results.


Report

No.	Sampl			Size	Height	Area	S/N Ratio	Comm
2	02_pla			666.6	478	5229	12.20	
3	04_pla			102.8	2814	71947	4.15	
4	06_plate1_1111_F01.fsa	Blue	948.9	101.1	2201	42685	5.79	
5	16_plate1_1111_H02.fsa	Blue	654.3	395.7	614	5529	15.48	
6	16_plate1_1111_H02.fsa	Green	653.2	396.8	332	2204	11.74	
7	18_plate1_1111_B03.fsa	Blue	113.0	937.0	410	1975	14.50	
8	18_plate1_1111_B03.fsa	Blue	466.2	583.8	2077	13689	48.74	
9	18_plate1_1111_B03.fsa	Green	462.1	587.9	585	5433	19.79	
10	27_plate1_1111_C04.fsa	Blue	84.6	965.4	297	3390	5.69	

Haplotype Analysis

Familial DNA fragment data is used for haplotype analysis in areas such as genetic disorder research and pre-implantation studies. Commercially available kits with markers for both autosomal and X-linked traits are available for diseases such as cystic fibrosis (CF) and Duchene Muscular Dystrophy (DMD). Traditionally, researchers determine the genotype of each family member, draw a pedigree diagram and assign the phase of the alleles where possible, based on the familial data of parents and child(ren).

Overview

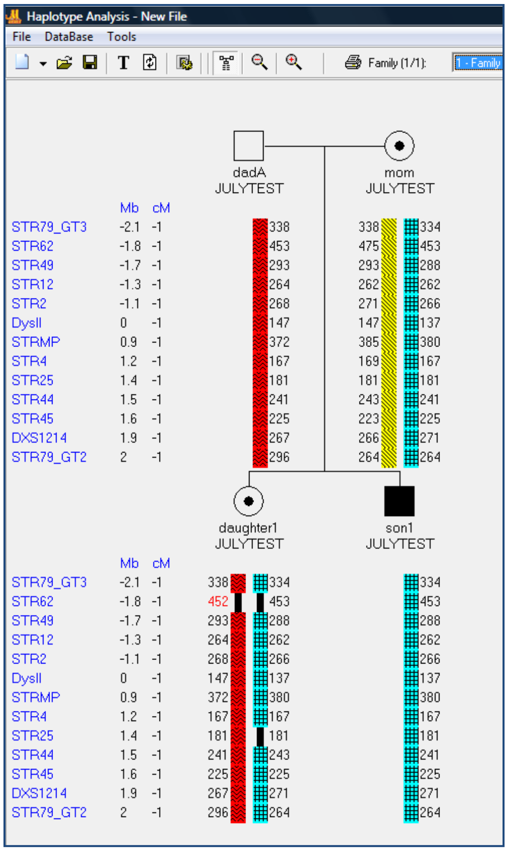
The Haplotype Analysis tool is directly linked to the main analysis screen in GeneMarker. After confirming the allele calls for each family member the data is opened in Haplotype Analysis where the program uses the allele calls of children and parents to assign phase of the alleles with a first order approximation. Whenever the alleles are informative for phase assignment a color/pattern bar is assigned to indicate most probable phase. Un-informative markers receive a solid black phase bar.

Features:

1. Follows Bennett *et al* nomenclature for pedigrees
2. X-linked and autosomal pedigree formats
3. Edit family and individual information
4. Displays markers, allele calls, personal information in pedigree
5. Control ordering of markers in pedigree by customizing panels
6. Automatically makes first order phase assignment based on parent/child(ren)
7. Edit capability for reassigning phase
8. Edit capability for crossover
9. Save complete pedigree (including edits) and re-opened to edit as more information becomes available
10. Allele conflicts flagged with red font, potential uniparental disomy is identified

Haplotype Analysis

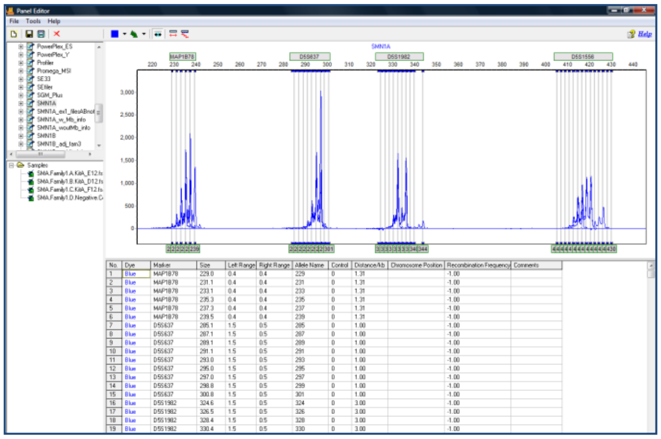
X-linked nomenclature, Assigned probable phase of alleles, Allele conflict/possible mutation in red font



	Mb	cM	dadA JULYTEST	mom JULYTEST	
STR79_GT3	-2.1	-1	338	338	334
STR62	-1.8	-1	453	475	453
STR49	-1.7	-1	293	293	288
STR12	-1.3	-1	264	262	262
STR2	-1.1	-1	268	271	266
DysII	0	-1	147	147	137
STRMP	0.9	-1	372	385	380
STR4	1.2	-1	167	169	167
STR25	1.4	-1	181	181	181
STR44	1.5	-1	241	243	241
STR45	1.6	-1	225	223	225
DXS1214	1.9	-1	267	266	271
STR79_GT2	2	-1	296	264	264

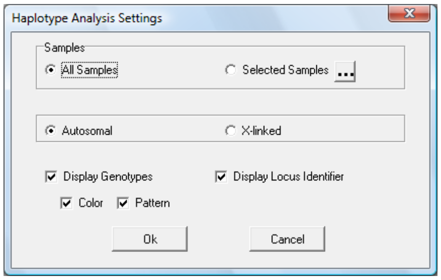
	Mb	cM	daughter1 JULYTEST		son1 JULYTEST
STR79_GT3	-2.1	-1	338	334	334
STR62	-1.8	-1	452	453	453
STR49	-1.7	-1	293	288	288
STR12	-1.3	-1	264	262	262
STR2	-1.1	-1	268	266	266
DysII	0	-1	147	137	137
STRMP	0.9	-1	372	380	380
STR4	1.2	-1	167	167	167
STR25	1.4	-1	181	181	181
STR44	1.5	-1	241	243	241
STR45	1.6	-1	225	225	225
DXS1214	1.9	-1	267	271	271
STR79_GT2	2	-1	296	264	264

Procedure



1. Open GeneMarker and upload raw data files
2. Select a Panel, Size Standard and **Fragment (Animal) Analysis**
3. Verify allele calls are accurate in the *Main Analysis* window and adjust parameter settings accordingly. **See Chapter 2 General Procedure.**
4. **Panel Editor** to input Mb distance and cM
   Mb distance is used to order the markers in the pedigree diagram and **cM** is used to report information on genetic distance entered by the analyst **See Chapter 5 Panel Editor.**

5. **Save Allele report as .txt file – select report save icon, marker table, horizontal, deselect extend heterozygous**
6. **Repeat steps 1-4 if a second kit or panel is required for a complete profile of the individual**
7. ***Applications*** drop down menu, select ***Haplotype Analysis***
8. *Tools* or parameter icon to select *Haplotype Analysis Settings*



Samples: ● All Samples ○ Selected Samples

● Autosomal ○ X-linked

☑ Display Genotypes ☑ Display Locus Identifier

☑ Color ☑ Pattern

Samples – All or selected Samples

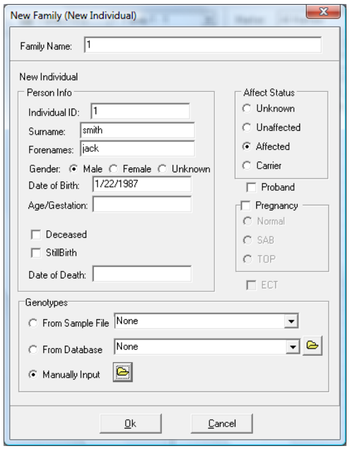
Autosomal – both haplobars are displayed
X-linked – males have one haplobar displayed

Display Genotypes – lists the allele calls under each node

Display Locus Identifier – lists marker names in the pedigree diagram

Color and pattern provides easily distinguishable haplobar for each individual
Pattern alone provides distinguishable haplobar for analysts with color impaired vision or for black and white printers

9. *File* drop down menu – *New Pedigree File*
10. Enter patient information
11. Enter Genotype results from one kit or two kits
    - ➢ From one kit – Select from Sample or From Database
    - ➢ From two kits – Select Manually Input and open file icon
        - o Navigate to the saved Allele Reports with the open folder icon
        - o Select kit 1 and kit 2 results for the individual
        - o Ok

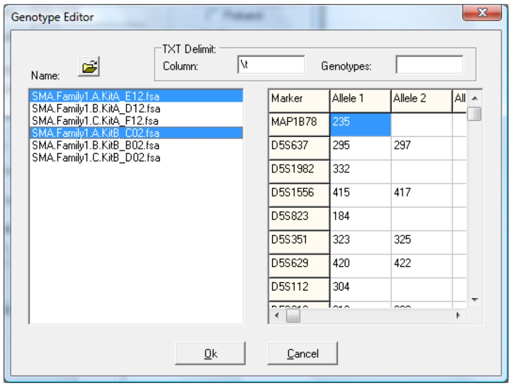


Family Name: 1

Individual ID: 1
Surname: smith
Forenames: jack
Gender: ● Male ○ Female ○ Unknown
Date of Birth: 1/22/1987
Age/Gestation:
☐ Deceased
☐ StillBirth
Date of Death:

Affect Status: ○ Unknown ○ Unaffected ● Affected ○ Carrier
☐ Proband
☐ Pregnancy ○ Normal ○ SAB ○ TOP
☐ ECT

Genotypes:
○ From Sample File None
○ From Database None
● Manually Input

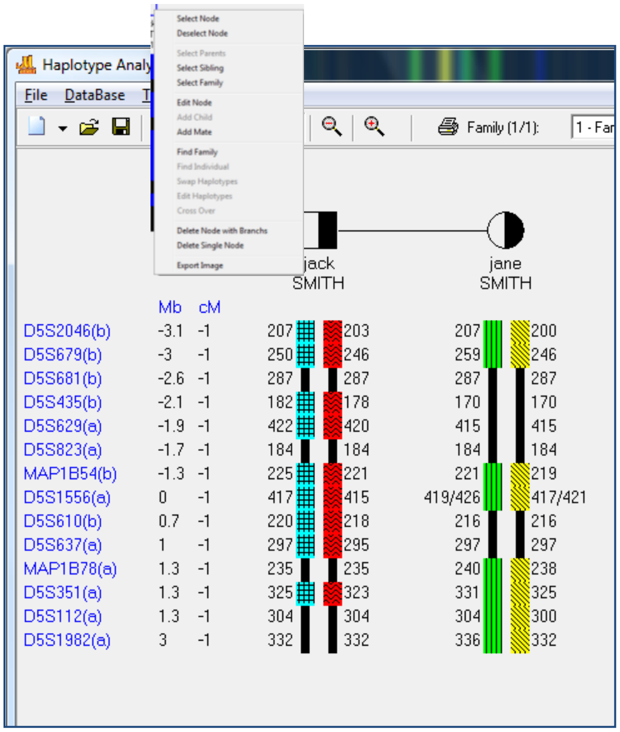


TXT Delimit:
Column: \t
Genotypes:

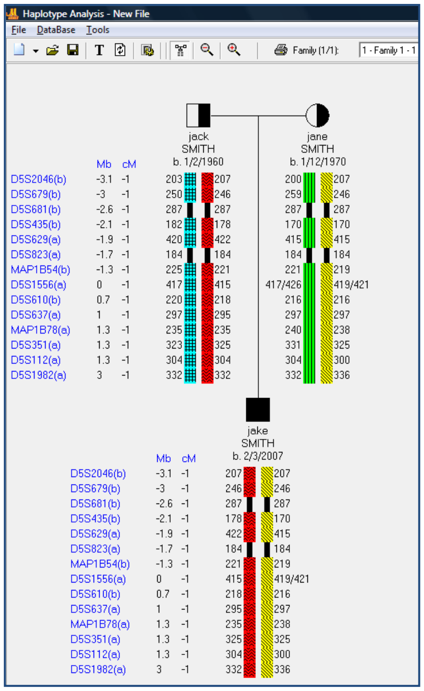
Name:
SMA.Family1.A.KitA_E12.fsa
SMA.Family1.B.KitA_D12.fsa
SMA.Family1.C.KitA_F12.fsa
SMA.Family1.A.KitB_C02.fsa
SMA.Family1.B.KitB_B02.fsa
SMA.Family1.C.KitB_D02.fsa

Marker	Allele 1	Allele 2	All
MAP1B78	235		
D5S637	295	297	
D5S1982	332		
D5S1556	415	417	
D5S823	184		
D5S351	323	325	
D5S629	420	422	
D5S112	304		

12. Right click on the node to activate a pop-up menu - edit or add mate and child(ren)


Haplotype Analy
File
DataBase
Family (1/1):
1 - Far
Select Node
Deselect Node
Select Parents
Select Sibling
Select Family
Edit Node
Add Child
Add Mate
Find Family
Find Individual
Swap Haplotypes
Edit Haplotypes
Cross Over
Delete Node with Branchs
Delete Single Node
Export Image

	Mb	cM	jack SMITH		jane SMITH	
D5S2046(b)	-3.1	-1	207	203	207	200
D5S679(b)	-3	-1	250	246	259	246
D5S681(b)	-2.6	-1	287	287	287	287
D5S435(b)	-2.1	-1	182	178	170	170
D5S629(a)	-1.9	-1	422	420	415	415
D5S823(a)	-1.7	-1	184	184	184	184
MAP1B54(b)	-1.3	-1	225	221	221	219
D5S1556(a)	0	-1	417	415	419/426	417/421
D5S610(b)	0.7	-1	220	218	216	216
D5S637(a)	1	-1	297	295	297	297
MAP1B78(a)	1.3	-1	235	235	240	238
D5S351(a)	1.3	-1	325	323	331	325
D5S112(a)	1.3	-1	304	304	304	300
D5S1982(a)	3	-1	332	332	336	332


Haplotype Analysis - New File
File
DataBase
Tools
Family (1/1):
1 - Family 1 - 1

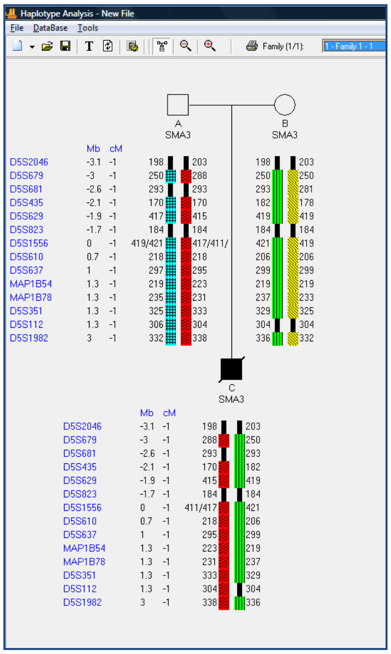
	Mb	cM	jack SMITH b. 1/2/1960		jane SMITH b. 1/12/1970	
D5S2046(b)	-3.1	-1	203	207	200	207
D5S679(b)	-3	-1	250	246	259	246
D5S681(b)	-2.6	-1	287	287	287	287
D5S435(b)	-2.1	-1	182	178	170	170
D5S629(a)	-1.9	-1	420	422	415	415
D5S823(a)	-1.7	-1	184	184	184	184
MAP1B54(b)	-1.3	-1	225	221	221	219
D5S1556(a)	0	-1	417	415	417/426	419/421
D5S610(b)	0.7	-1	220	218	216	216
D5S637(a)	1	-1	297	295	297	297
MAP1B78(a)	1.3	-1	235	235	240	238
D5S351(a)	1.3	-1	323	325	331	325
D5S112(a)	1.3	-1	304	304	304	300
D5S1982(a)	3	-1	332	332	332	336

	Mb	cM	jake SMITH b. 2/3/2007	
D5S2046(b)	-3.1	-1	207	207
D5S679(b)	-3	-1	246	246
D5S681(b)	-2.6	-1	287	287
D5S435(b)	-2.1	-1	178	170
D5S629(a)	-1.9	-1	422	415
D5S823(a)	-1.7	-1	184	184
MAP1B54(b)	-1.3	-1	221	219
D5S1556(a)	0	-1	415	419/421
D5S610(b)	0.7	-1	218	216
D5S637(a)	1	-1	295	297
MAP1B78(a)	1.3	-1	235	238
D5S351(a)	1.3	-1	325	325
D5S112(a)	1.3	-1	304	300
D5S1982(a)	3	-1	332	336

Automatically refreshes and re-assigns parental phase with addition of child(ren) information

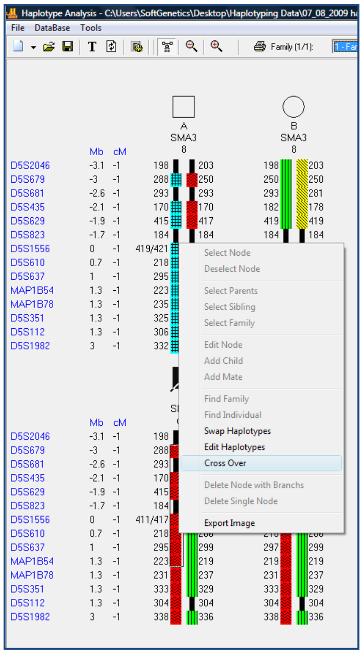
13. Review phase assignment and make edits – right click and select swap or edit haplotype or cross-over

To edit crossover – use the mouse to draw a box around the appropriate section of the haplobar


Haplotype Analysis - New File
File
DataBase
Tools
Family (1/1):
1 - Family 1 - 1

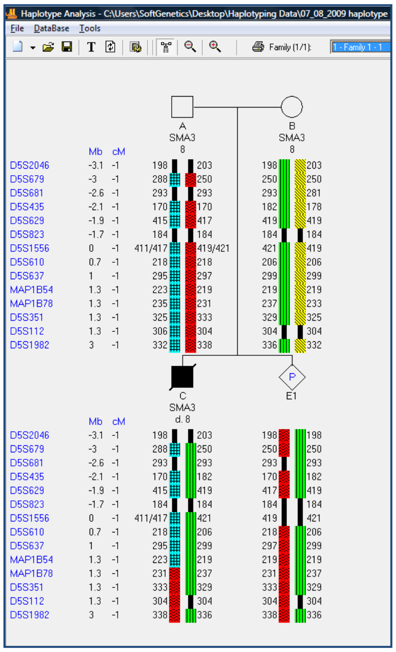
	Mb	cM	A SMA3		B SMA3	
D5S2046	-3.1	-1	198	203	198	203
D5S679	-3	-1	250	288	250	250
D5S681	-2.6	-1	293	293	293	281
D5S435	-2.1	-1	170	170	182	178
D5S629	-1.9	-1	417	415	419	419
D5S823	-1.7	-1	184	184	184	184
D5S1556	0	-1	419/421	417/411/	421	419
D5S610	0.7	-1	218	218	206	206
D5S637	1	-1	297	295	299	299
MAP1B54	1.3	-1	219	223	219	219
MAP1B78	1.3	-1	235	231	237	233
D5S351	1.3	-1	325	333	329	325
D5S112	1.3	-1	306	304	304	304
D5S1982	3	-1	332	338	336	332

	Mb	cM	C SMA3	
D5S2046	-3.1	-1	198	203
D5S679	-3	-1	288	250
D5S681	-2.6	-1	293	293
D5S435	-2.1	-1	170	182
D5S629	-1.9	-1	415	419
D5S823	-1.7	-1	184	184
D5S1556	0	-1	411/417	421
D5S610	0.7	-1	218	206
D5S637	1	-1	295	299
MAP1B54	1.3	-1	223	219
MAP1B78	1.3	-1	231	237
D5S351	1.3	-1	333	329
D5S112	1.3	-1	304	304
D5S1982	3	-1	338	336


Haplotype Analysis - C:\Users\SoftGenetics\Desktop\Haplotyping Data\07_08_2009 ha
File
DataBase
Tools
Family (1/1):
1 - Far
Select Node
Deselect Node
Select Parents
Select Sibling
Select Family
Edit Node
Add Child
Add Mate
Find Family
Find Individual
Swap Haplotypes
Edit Haplotypes
Cross Over
Delete Node with Branchs
Delete Single Node
Export Image

	Mb	cM	A SMA3 8		B SMA3 8	
D5S2046	-3.1	-1	198	203	198	203
D5S679	-3	-1	288	250	250	250
D5S681	-2.6	-1	293	293	293	281
D5S435	-2.1	-1	170	170	182	178
D5S629	-1.9	-1	415	417	419	419
D5S823	-1.7	-1	184	184	184	184
D5S1556	0	-1	419/421			
D5S610	0.7	-1	218			
D5S637	1	-1	295			
MAP1B54	1.3	-1	223			
MAP1B78	1.3	-1	235			
D5S351	1.3	-1	325			
D5S112	1.3	-1	306			
D5S1982	3	-1	332			

	Mb	cM				
D5S2046	-3.1	-1	198			
D5S679	-3	-1	288			
D5S681	-2.6	-1	293			
D5S435	-2.1	-1	170			
D5S629	-1.9	-1	415			
D5S823	-1.7	-1	184			
D5S1556	0	-1	411/417			
D5S610	0.7	-1	218			
D5S637	1	-1	295	299	297	299
MAP1B54	1.3	-1	223	219	219	219
MAP1B78	1.3	-1	231	237	231	237
D5S351	1.3	-1	333	329	333	329
D5S112	1.3	-1	304	304	304	304
D5S1982	3	-1	338	336	338	336


Haplotype Analysis - C:\Users\SoftGenetics\Desktop\Haplotyping Data\07_08_2009 haplotype
File
DataBase
Tools
Family (1/1):
1 - Family 1 - 1
P

	Mb	cM	A SMA3 8		B SMA3 8	
D5S2046	-3.1	-1	198	203	198	203
D5S679	-3	-1	288	250	250	250
D5S681	-2.6	-1	293	293	293	281
D5S435	-2.1	-1	170	170	182	178
D5S629	-1.9	-1	415	417	419	419
D5S823	-1.7	-1	184	184	184	184
D5S1556	0	-1	411/417	419/421	421	419
D5S610	0.7	-1	218	218	206	206
D5S637	1	-1	295	297	299	299
MAP1B54	1.3	-1	223	219	219	219
MAP1B78	1.3	-1	235	231	237	233
D5S351	1.3	-1	325	333	329	325
D5S112	1.3	-1	306	304	304	304
D5S1982	3	-1	332	338	336	332

	Mb	cM	C SMA3 d. 8		E1	
D5S2046	-3.1	-1	198	203	198	198
D5S679	-3	-1	288	250	250	250
D5S681	-2.6	-1	293	293	293	293
D5S435	-2.1	-1	170	182	170	182
D5S629	-1.9	-1	415	419	417	419
D5S823	-1.7	-1	184	184	184	184
D5S1556	0	-1	411/417	421	419	421
D5S610	0.7	-1	218	206	218	206
D5S637	1	-1	295	299	297	299
MAP1B54	1.3	-1	223	219	219	219
MAP1B78	1.3	-1	231	237	231	237
D5S351	1.3	-1	333	329	333	329
D5S112	1.3	-1	304	304	304	304
D5S1982	3	-1	338	336	338	336

Icons

New Pedigree File
Select *New Pedigree File* to create a new pedigree with multiple families OR select *New Family* to add a family to the pedigree file. Enter the first family member's information into the *New Family (New Individual)* box and click **OK** to create a new *Pedigree Tree*.

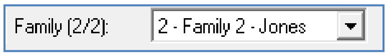
Open Pedigree File
Launches the Load Pedigree File box. Select a PED or PRE file to upload (the SMP file will automatically upload) and click OK.

Save Pedigree File
Launches the *Save Pedigree File* box. Enter filename and change directory to save the Pedigree Files (PRE, SMP, DAT).

Update Sample Data
Select to refresh the Mendelian inheritance calculation after a node or allele is edited and after selection a different family when the *'show genotype'* display is used

Haplotype Analysis Parameters
Launches the *Haplotype Anaylysis Settings* box. Options for selected samples or all samples, Autosomal or X-linked analysis and display options

Show Genotype
Toggle between *Displaying and Not Displaying the Genotypes* of the selected node


Family (2/2): 2 - Family 2 - Jones

Family
Select a family from the currently uploaded pedigree file to view and edit.

Marker: 12 - TPOX

Marker
Select a Marker or Locus to view in the *Electropherogram Charts*.

Show Color
Allows the user to select all colors to view, hide all colors, or choose a single dye layer. Choose a single dye by single left mouse clicking on the icon.

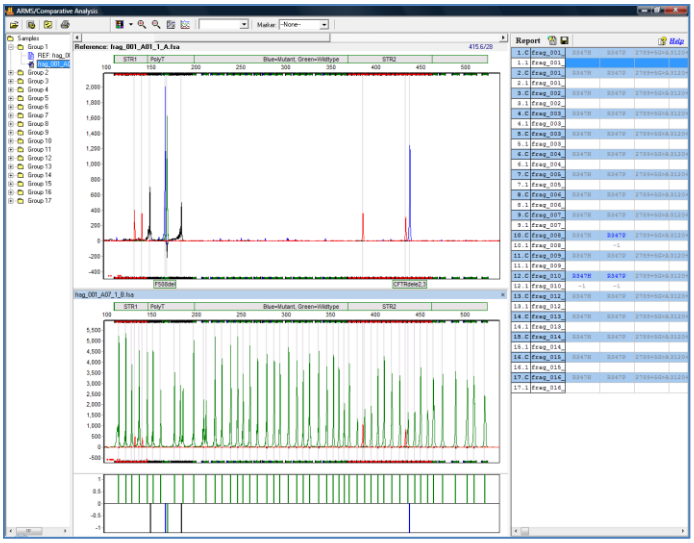
Zoom In
Use the icon to zoom in on the image, or hold down the left mouse button and draw a box, from the top left corner to bottom right corner, around the area you wish to zoom in.

Zoom Out
Use the icon to zoom out on the image, or hold down the left mouse button and draw a box, from the bottom right corner to top left corner.

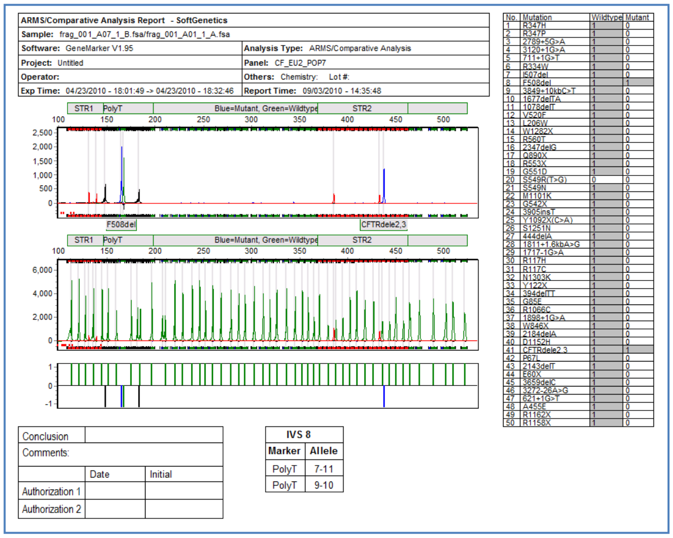
ARMS/Comparative Analysis for Cystic Fibrosis Analysis

Overview

This analysis type allows comparison of pairs of files for a given individual within the same project; includes: electropherograms trace comparison and allele report in a patient report format. For example, if kits use one dye in a multiplex for most of the wild type alleles in one multiplex and a second dye in a second multiplex for the mutant alleles (as is found in GeneProbe®EU-2 for cystic fibrosis), the ARMS//Comparative Analysis application provides an accurate, concise comparison of the two files for the individual.



Trace Comparison with Linked Report Table



ARMS/Comparative Analysis Report - SoftGenetics	
Sample: frag_001_A07_1_B.fsa/frag_001_A01_1_A.fsa	
Software: GeneMarker V1.95	**Analysis Type:** ARMS/Comparative Analysis
Project: Untitled	**Panel:** CF_EU2_POP7
Operator:	**Others:** Chemistry: Lot #:
Exp Time: 04/23/2010 - 18:01:49 -> 04/23/2010 - 18:32:46	**Report Time:** 09/03/2010 - 14:35:48

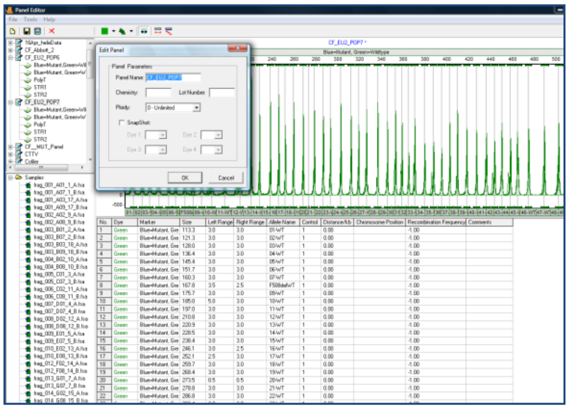
Conclusion		
Comments:		
	Date	Initial
Authorization 1		
Authorization 2		

IVS 8	
Marker	**Allele**
PolyT	7-11
PolyT	9-10

No.	Mutation	Wildtype	Mutant
1	R347H	1	0
2	R347P	1	0
3	2789+5G>A	1	0
4	3120+1G>A	1	0
5	711+1G>T	1	0
6	R334W	1	0
7	I507del	1	0
8	F508del	1	1
9	3849+10kbC>T	1	0
10	1677delTA	1	0
11	1078delT	1	0
12	V520F	1	0
13	L206W	1	0
14	W1282X	1	0
15	R560T	1	0
16	2347delG	1	0
17	Q890X	1	0
18	R553X	1	0
19	G551D	1	0
20	S549R(T>G)	0	0
21	S549N	1	0
22	M1101K	1	0
23	G542X	1	0
24	3905insT	1	0
25	Y1092X(C>A)	1	0
26	S1251N	1	0
27	444delA	1	0
28	1811+1.6kbA>G	1	0
29	1717-1G>A	1	0
30	R117H	1	0
31	R117C	1	0
32	N1303K	1	0
33	Y122X	1	0
34	394delTT	1	0
35	G85E	1	0
36	R1066C	1	0
37	1898+1G>A	1	0
38	W846X	1	0
39	2184delA	1	0
40	D1152H	1	0
41	CFTRdele2,3	1	1
42	P67L	1	0
43	2143delT	1	0
44	E60X	1	0
45	3659delC	1	0
46	3272-26A>G	1	0
47	621+1G>T	1	0
48	A455E	1	0
49	R1162X	1	0
50	R1158X	1	0

Final Report with Trace Comparison, Report Tables, Header and Comments Section

Procedure



1. Open GeneMarker and upload raw data files
2. Select a Panel, Size Standard and **Fragment (Animal) Analysis**
3. Use the default settings in the second and third screen of the Run Wizard, or customize as needed See Chapter 2 General Procedure
4. Verify size calls are accurate in the *Main Analysis* window and adjust parameter settings accordingly. **See Chapter 2 General Procedure.**
5. **Tools →Panel Editor** to import panel and enter 0 in the Control Column for alleles that are Mutant Alleles and 1 for Wild Type Alleles**.** **See Chapter 5 Panel Editor.**
6. **Run Wizard** with the panel and review allele calls.
7. **File →Save Project**
8. **Project → Apply Sample Grouping** to pair both files for an individual (See Chapter 9 Additional Tools.)
9. ***Applications*** drop down menu, select ***ARMS/ComarativeAnalysis*** and select the appropriate option. Select Mutant + Wildtype to open the Trace Comparison Application of grouped files. Select Mutant Only for analysis of a single file per individual to go directly to the print report preview.
10. *Select the Tools* or parameter icon to select ARMS *Comparative/Analysis* Parameters
11. Select the desired Parameter Options
12. Review analysis and select Print Preview or Print

Icons and Functions

🗎	Comparative Analysis Parameters

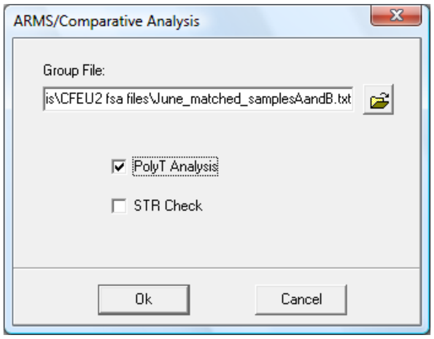
Launches the dialog box to select the analysis parameters

Layout Settings
Select the Comparative Analysis Display Settings

Printer
Select the Printing Options for the final report. Print from this screen or select Print Preview

What to Expect

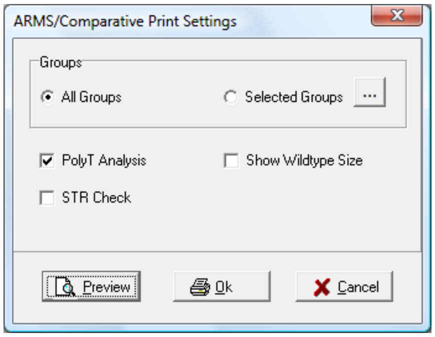
The Header in the final report obtains Institution and Operator information from User Management. All other information in the header is from the Panel and from the Project Parameters. The Allele calls are entered into the Report Table from the Project. The Control Column of the panel editor determines whether the allele call of the blue and green channels is placed in the Wildtype or Mutant column of the report table. Some chemistries provide the option to amplify and report Poly T and unique STR information. These settings are deselected in the default settings. The analysis parameters and print settings in GeneMarker allow the analyst to determine whether or not to analyze and report these results.



Group File: is\CFEU2 fsa files\June_matched_samplesAandB.txt

☑ PolyT Analysis

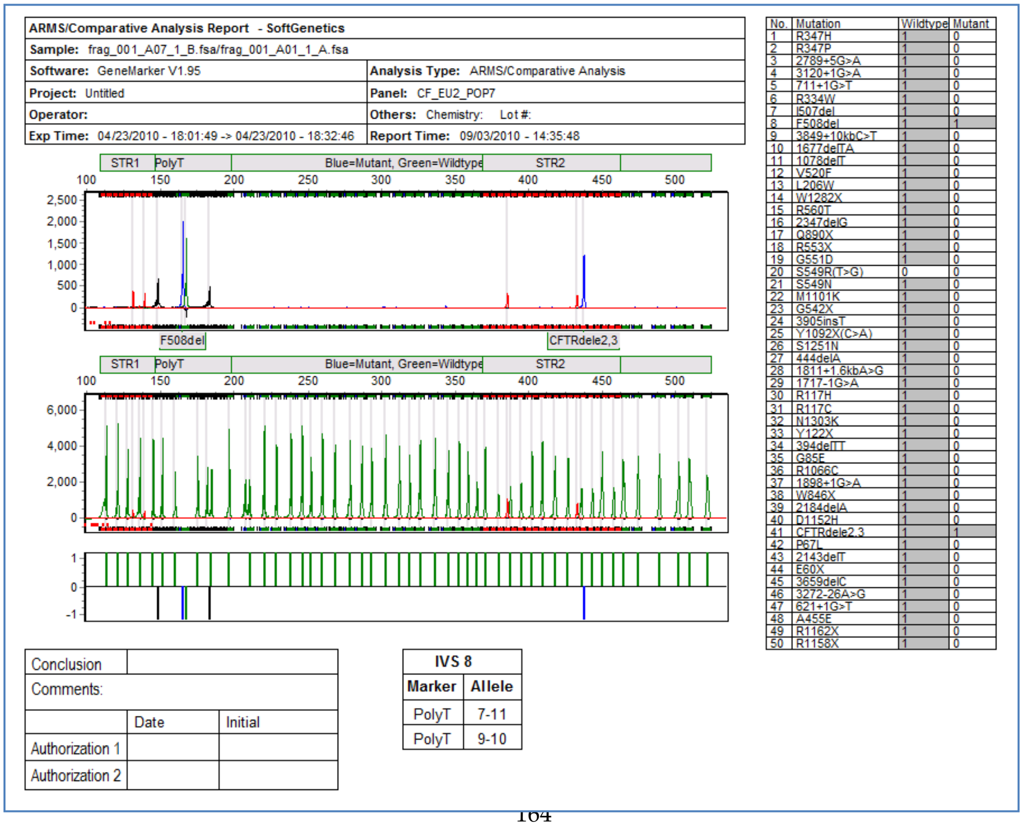
☐ STR Check



Groups: ● All Groups ○ Selected Groups

☑ PolyT Analysis ☐ Show Wildtype Size

☐ STR Check



ARMS/Comparative Analysis Report - SoftGenetics	
Sample: frag_001_A07_1_B.fsa/frag_001_A01_1_A.fsa	
Software: GeneMarker V1.95	**Analysis Type:** ARMS/Comparative Analysis
Project: Untitled	**Panel:** CF_EU2_POP7
Operator:	**Others:** Chemistry: Lot #:
Exp Time: 04/23/2010 - 18:01:49 -> 04/23/2010 - 18:32:46	**Report Time:** 09/03/2010 - 14:35:48

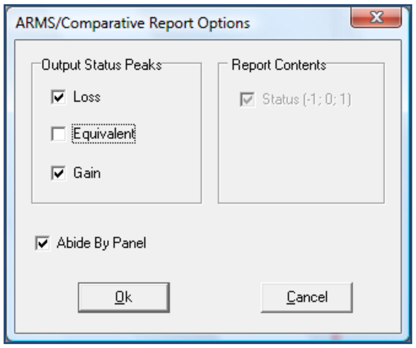
No.	Mutation	Wildtype	Mutant
1	R347H	1	0
2	R347P	1	0
3	2789+5G>A	1	0
4	3120+1G>A	1	0
5	711+1G>T	1	0
6	R334W	1	0
7	I507del	1	0
8	F508del	1	1
9	3849+10kbC>T	1	0
10	1677delTA	1	0
11	1078delT	1	0
12	V520F	1	0
13	L206W	1	0
14	W1282X	1	0
15	R560T	1	0
16	2347delG	1	0
17	Q890X	1	0
18	R553X	1	0
19	G551D	1	0
20	S549R(T>G)	0	0
21	S549N	1	0
22	M1101K	1	0
23	G542X	1	0
24	3905insT	1	0
25	Y1092X(C>A)	1	0
26	S1251N	1	0
27	444delA	1	0
28	1811+1.6kbA>G	1	0
29	1717-1G>A	1	0
30	R117H	1	0
31	R117C	1	0
32	N1303K	1	0
33	Y122X	1	0
34	394delTT	1	0
35	G85E	1	0
36	R1066C	1	0
37	1898+1G>A	1	0
38	W846X	1	0
39	2184delA	1	0
40	D1152H	1	0
41	CFTRdele2,3	1	1
42	P67L	1	0
43	2143delT	1	0
44	E60X	1	0
45	3659delC	1	0
46	3272-26A>G	1	0
47	621+1G>T	1	0
48	A455E	1	0
49	R1162X	1	0
50	R1158X	1	0

Conclusion		
Comments:		
	Date	Initial
Authorization 1		
Authorization 2		

IVS 8	
Marker	**Allele**
PolyT	7-11
PolyT	9-10

The report table contains a 1 for any allele where a peak is detected and a 0 if no peak is detected. This report is an example of on individual heterozygous for Cystic Fibrosis alleles F508del and CFTRdele2_3 with heterozygosity in the polyT region in the 7 and 9 repeats.

Reports and Printing


ARMS/Comparative Report Options

Output Status Peaks
☑ Loss
☐ Equivalent
☑ Gain

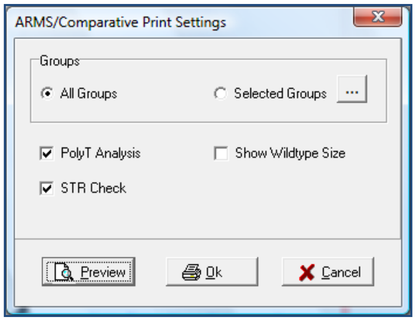
Report Contents
☑ Status (-1; 0; 1)

☑ Abide By Panel

Report

Launches the Report table options dialog box

Save Icon
Save the Report table as an .xls or .txt file

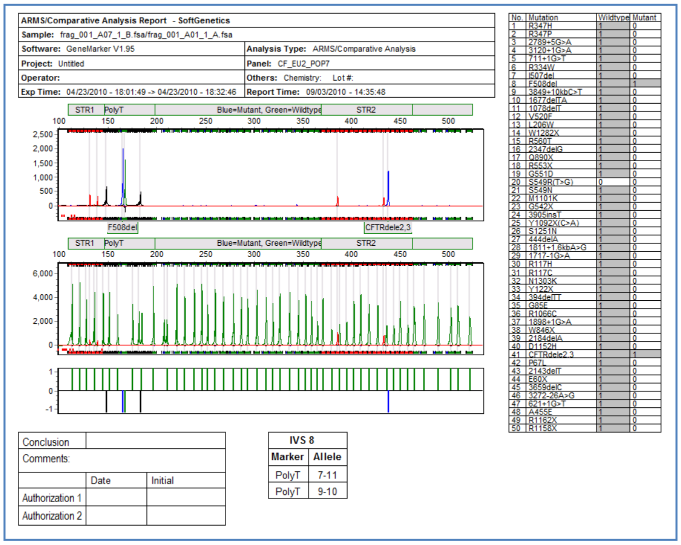

ARMS/Comparative Print Settings

Groups
● All Groups ○ Selected Groups ...

☑ PolyT Analysis ☐ Show Wildtype Size
☑ STR Check

Preview
Ok
Cancel

Printer Icon
Provides Print Settings – if the polyT (Ned) information is not required it may be deselected

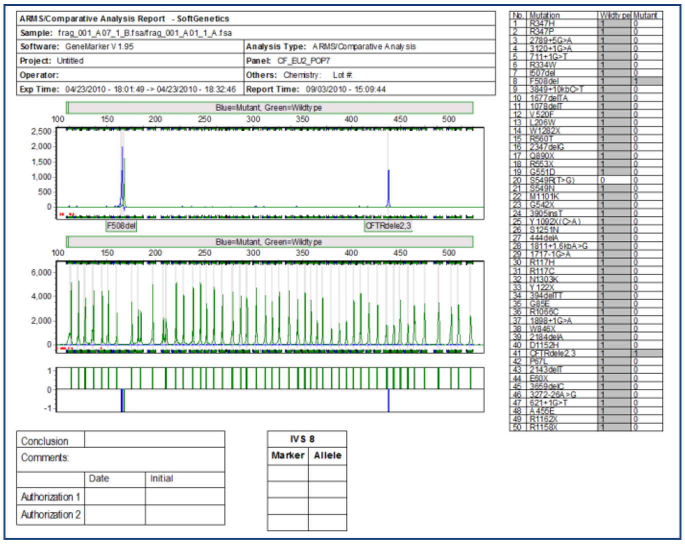

ARMS/Comparative Analysis Report - SoftGenetics	
Sample: frag_001_A07_1_B.fsa/frag_001_A01_1_A.fsa	
Software: GeneMarker V1.95	**Analysis Type:** ARMS/Comparative Analysis
Project: Untitled	**Panel:** CF_EU2_POP7
Operator:	**Others:** Chemistry: Lot #:
Exp Time: 04/23/2010 - 18:01:49 -> 04/23/2010 - 18:32:46	**Report Time:** 09/03/2010 - 14:35:48

STR1
PolyT
Blue=Mutant, Green=Wildtype
STR2
100
150
200
250
300
350
400
450
500
2,500
2,000
1,500
1,000
500
0
F508del
CFTRdele2,3
STR1
PolyT
Blue=Mutant, Green=Wildtype
STR2
6,000
4,000
2,000
0
1
0
-1

No.	Mutation	Wildtype	Mutant
1	R347H	1	0
2	R347P	1	0
3	2789+5G>A	1	0
4	3120+1G>A	1	0
5	711+1G>T	1	0
6	R334W	1	0
7	I507del	1	0
8	F508del	1	1
9	3849+10kbC>T	1	0
10	1677delTA	1	0
11	1078delT	1	0
12	V520F	1	0
13	L206W	1	0
14	W1282X	1	0
15	R560T	1	0
16	2347delG	1	0
17	Q890X	1	0
18	R553X	1	0
19	G551D	1	0
20	S549R(T>G)	0	0
21	S549N	1	0
22	M1101K	1	0
23	G542X	1	0
24	3905insT	1	0
25	Y1092X(C>A)	1	0
26	S1251N	1	0
27	444delA	1	0
28	1811+1.6kbA>G	1	0
29	1717-1G>A	1	0
30	R117H	1	0
31	R117C	1	0
32	N1303K	1	0
33	Y122X	1	0
34	394delTT	1	0
35	G85E	1	0
36	R1066C	1	0
37	1898+1G>A	1	0
38	W846X	1	0
39	2184delA	1	0
40	D1152H	1	0
41	CFTRdele2,3	1	1
42	P67L	1	0
43	2143delT	1	0
44	E60X	1	0
45	3659delC	1	0
46	3272-26A>G	1	0
47	621+1G>T	1	0
48	A455E	1	0
49	R1162X	1	0
50	R1158X	1	0

Conclusion		
Comments:		
	Date	Initial
Authorization 1		
Authorization 2		

IVS 8	
Marker	**Allele**
PolyT	7-11
PolyT	9-10


ARMS/Comparative Analysis Report - SoftGenetics	
Sample: frag_001_A07_1_B.fsa/frag_001_A01_1_A.fsa	
Software: GeneMarker V1.95	**Analysis Type:** ARMS/Comparative Analysis
Project: Untitled	**Panel:** CF_EU2_POP7
Operator:	**Others:** Chemistry: Lot #:
Exp Time: 04/23/2010 - 18:01:49 -> 04/23/2010 - 18:32:46	**Report Time:** 09/03/2010 - 15:09:44

Blue=Mutant, Green=Wildtype
100
150
200
250
300
350
400
450
500
2,500
2,000
1,500
1,000
500
0
F508del
CFTRdele2,3
Blue=Mutant, Green=Wildtype
6,000
4,000
2,000
0
1
0
-1

No.	Mutation	Wildtype	Mutant
1	R347H	1	0
2	R347P	1	0
3	2789+5G>A	1	0
4	3120+1G>A	1	0
5	711+1G>T	1	0
6	R334W	1	0
7	I507del	1	0
8	F508del	1	1
9	3849+10kbC>T	1	0
10	1677delTA	1	0
11	1078delT	1	0
12	V520F	1	0
13	L206W	1	0
14	W1282X	1	0
15	R560T	1	0
16	2347delG	1	0
17	Q890X	1	0
18	R553X	1	0
19	G551D	1	0
20	S549R(T>G)	0	0
21	S549N	1	0
22	M1101K	1	0
23	G542X	1	0
24	3905insT	1	0
25	Y1092X(C>A)	1	0
26	S1251N	1	0
27	444delA	1	0
28	1811+1.6kbA>G	1	0
29	1717-1G>A	1	0
30	R117H	1	0
31	R117C	1	0
32	N1303K	1	0
33	Y122X	1	0
34	394delTT	1	0
35	G85E	1	0
36	R1066C	1	0
37	1898+1G>A	1	0
38	W846X	1	0
39	2184delA	1	0
40	D1152H	1	0
41	CFTRdele2,3	1	1
42	P67L	1	0
43	2143delT	1	0
44	E60X	1	0
45	3659delC	1	0
46	3272-26A>G	1	0
47	621+1G>T	1	0
48	A455E	1	0
49	R1162X	1	0
50	R1158X	1	0

Conclusion		
Comments:		
	Date	Initial
Authorization 1		
Authorization 2		

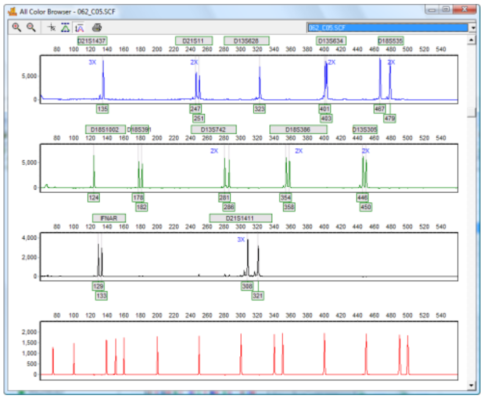
IVS 8	
Marker	**Allele**

The same patient report, the left one has all fields selected and the report on the right has only the FAM and VIC dyes selected.

Chapter 8 Additional Tools

Chapter 8 Additional Tools
Browse By All Colors
Overlay View
Merge Projects
Macromolecules
File Conversion
Filename Group Editor
Output Trace Data
Project Comparison
Convert TXT to Binary
Export Electropherogram

Browse By All Colors


All Color Browser - 062_C05.SCF
062_C05.SCF
D21S1437
D21S11
D13S628
D13S634
D18S535
D18S1002
D18S391
D13S742
D18S386
D13S305
FNAR
D21S1411
2X
3X
5,000
4,000
2,000
2,000
1,500
1,000
500
0

Click the **Browse by All Colors** icon in the *Main Analysis* window toolbar.

Navigation and peak editing options in the *All Color Browser* is similar to the *Main Analysis* window. **See Chapter 3 Main Analysis Overview.**

To scroll through samples in the *All Color Browser*, click the drop-down menu in the upper right corner and select a sample from the list. Once a sample is selected in the drop-down menu, you can use the **Up/Down Arrow** keys to scroll through samples.

Icons and Functions

Zoom In/Out
Use these icons to increase/decrease the zoom aspect of the electropherograms.

Show/Hide Mouse Cross Lines
When selected, x and y-axis grid lines will appear at the tip of the mouse cursor along with the basepair size and RFU value of the mouse cursor position.

Show/Hide Bin Ranges
When selected, the Bin brackets at the top and bottom of the electropherogram trace will appear.

Auto Scale Markers
When selected, the RFU intensities of low peaks are adjusted to match the intensity of the highest peak in the dye color. When low peaks are increased, the intensity magnification factor is noted in the Marker (2X – 8X).

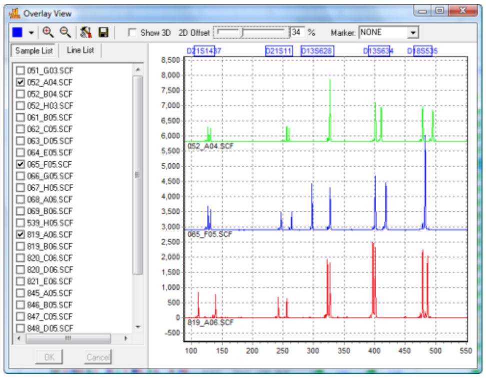
Print
Opens the *Print Report* settings box. **See Chapter 6 Reports and Printing**.

Overlay View

Applications → Overlay View

The *Overlay View* tool was developed as an easy way to compare several sample traces at once.

Procedure


Overlay View
Show 3D
2D Offset
34 %
Marker: NONE
Sample List
Line List
☐ 051_G03.SCF
☑ 052_A04.SCF
☐ 052_B04.SCF
☐ 052_H03.SCF
☐ 061_B05.SCF
☐ 062_C05.SCF
☐ 063_D05.SCF
☐ 064_E05.SCF
☑ 065_F05.SCF
☐ 066_G05.SCF
☐ 067_H05.SCF
☐ 068_A06.SCF
☐ 069_B06.SCF
☐ 539_H05.SCF
☑ 819_A06.SCF
☐ 819_B06.SCF
☐ 820_C06.SCF
☐ 820_D06.SCF
☐ 821_E06.SCF
☐ 845_A05.SCF
☐ 846_B05.SCF
☐ 847_C05.SCF
☐ 848_D05.SCF
OK
Cancel
D21S1437
D21S11
D13S628
D13S634
D18S535
052_A04.SCF
065_F05.SCF
819_A06.SCF
8,500
8,000
7,500
7,000
6,500
6,000
5,500
5,000
4,500
4,000
3,500
3,000
2,500
2,000
1,500
1,000
500
0
-500
100
150
200
250
300
350
400
450
500
550

1. Select several samples from the *Sample List* by placing a check mark in the empty box to the left of the filename.
2. Click **OK**
3. The traces will appear in the window to the right.
4. Slide the *2D Offset* bar to the right to de-convolute the traces.
5. Select the *Line List* tab to open the list of traces present in the viewer. Select any trace to bold the trace line.
6. Select a marker from the drop-down list to view one marker at a time.
7. Select *Show 3D* to see the traces in a three dimensional view.

Icons and Functions

Dye Color
Single click to scroll through the dye colors

Zoom In/Out
Single click to zoom in or out on the center of the *Trace View* window.

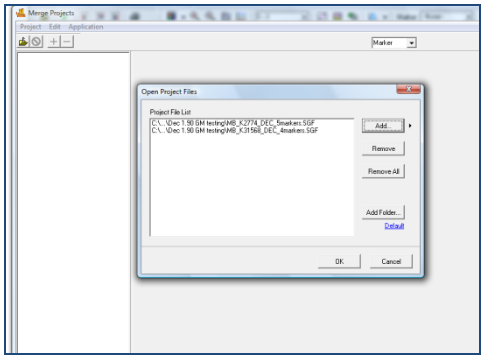
Tools
All viewing options are selected by default. Unselect these options to change the *Trace View* settings.

Save
Save the *Trace View* image as a Bitmap (.bmp) file.

Merge Projects

The Merge Projects Application is available directly from the opening GeneMarker Screen or from the Tools drop-down menu. After each project is analyzed with the appropriate panel the results may be combined using this tool. A common challenge for Kinship calculations or cluster analysis in animal and plant populations is obtaining enough informative markers in one multiplex. Overlapping marker ranges and/or incompatible chemistry make it necessary to run the same samples multiple times with different sets of marker primers. Human disease diagnostic research also my require more than one kit (multiplex) to obtain the full range of allele calls as with Duchenne's Muscular Dystrophy or Cystic Fibrosis.

By following a naming convention where the individual identifier and the panel identifiers are consistent, researchers can combine the individual results from multiple panel analyses into one report, providing a more complete genotype for each sample. This merged report may be imported into other special applications (such as Clustering Analysis or Relationship Testing) to improve the robustness of the results by including information from more markers.

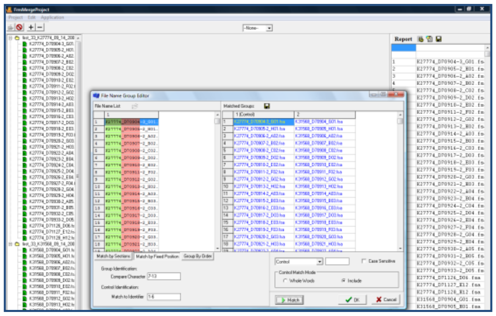


C:\...\Dec 1.90 GM testing\MB_K2774_DEC_5markers.SGF
C:\...\Dec 1.90 GM testing\MB_K31568_DEC_4markers.SGF

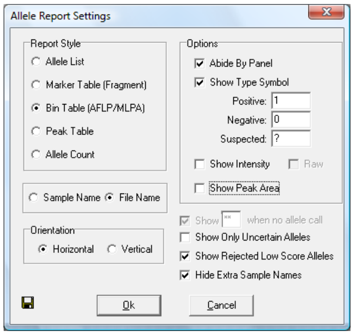
Procedure

1. Save each project for a set of individual files (Please see Chapter 2 General Procedure) **Note**: Allele editing and resolution of any flagged allele calls must be done prior to saving each project and importing results into the Merge Project Tool.
2. Open Merge Projects from the Tool Drop-down menu or the Open Folder icon
3. Projects → Merge Projects →Add project files →OK
4. Report Grouping icon activates the Grouping screen
5. Match by Section, Fixed Position or Group Order →Match →OK
   Note: To simplify this task separate the group identifier and the exact control identifier by an underscore or have the same number of spaces for the individual and group identifiers in a file name. For example – if the individual's identification number is 12345 and it will be analyzed with panel XYZ and panel ABC separate the sections by an underscore when applying the sample to the genetic analyzer.

Project with XYZ	Project with ABC
12345_XYZ_A01	12345_ABC_A01
12346_XYZ_A02	12346_ABC_A02
12347_XYZ_A03	12347_ABC_A03



6. Use the Report Icon to activate the Allele Report Settings
7. Select appropriate format :
   For example: Select Bin Table and deselect Show Peak Area for combined reports that will be used in Cluster Analysis or Select Marker Table (Fragment) for combined reports that will be used in Relationship Testing
8. Save the merged project report as a .txt file. Note: The file name will automatically read from the current project. Users may want to use a specific file name for the merged project report.



Allele Report Settings

Report Style
○ Allele List
○ Marker Table (Fragment)
● Bin Table (AFLP/MLPA)
○ Peak Table
○ Allele Count

○ Sample Name ● File Name

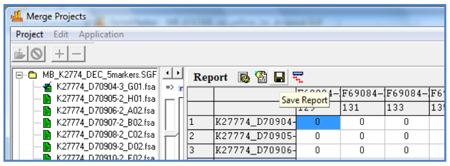
Orientation
● Horizontal ○ Vertical

Options
☑ Abide By Panel
☑ Show Type Symbol
Positive: 1
Negative: 0
Suspected: ?
☐ Show Intensity ☐ Raw
☐ Show Peak Area

☑ Show ** when no allele call
☐ Show Only Uncertain Alleles
☑ Show Rejected Low Score Alleles
☑ Hide Extra Sample Names

Ok Cancel

Icons and Functions



MB_K2774_DEC_5markers.SGF
K27774_D70904-3_G01.fsa
K27774_D70905-2_H01.fsa
K27774_D70906-2_A02.fsa
K27774_D70907-2_B02.fsa
K27774_D70908-2_C02.fsa
K27774_D70909-2_D02.fsa
K27774_D70910-2_E02.fsa

		F69084-	F69084-	F69084-	F6
		129	131	133	13
1	K27774_D70904-	0	0	0	
2	K27774_D70905-	0	0	0	
3	K27774_D70906-	0	0	0	

Load Files
Activates a dialog box to open the project files to allow merging of allele reports

Renaming Tool
This icon in the upper tool bar activates a renaming tool. Use this function to transiently rename files that cannot be grouped by any of the methods in step 5 above.

Report Grouping
Activates the report grouping to allow combining allele reports of the same samples that were produced from different multiplexes

Allele Report Settings
Allows selection of the appropriate allele report settings for each analysis type

Save
Save the combined allele report as a .txt file for use in applications such as Cluster Analysis or Relationship Testing

Functions

Match by Sections

Automatically separates the sample filenames into groups based on the specified *Section Separators*.

Group Identification: Identifies how to match the filenames into groups based on the section entered into the *Compare by Section* field. The section of the filename specified will be highlighted red in the *File Name List*.

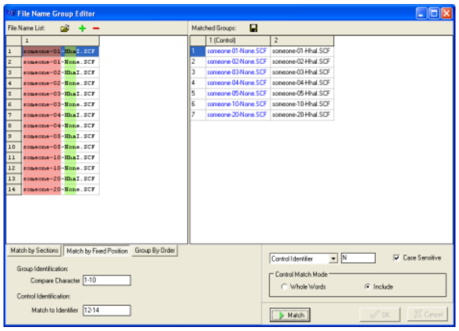
Control Identification: Identifies which section of the filename contains the reference vs. sample information based on the section number entered in the *Match to Identifier by Section* field. The section of the filename specified will be highlighted green in the *File Name List*.

Match by Fixed Position

Allows the user to manually identify the characters of the filename for grouping the samples. *Section Separators* like "_ , -" are counted as individual characters.

Group Identification: Enter the number of the beginning and ending character to identify how to group the samples. The section of the filename specified will be highlighted red in the *File Name List*.

Control Identification: Enter the number of the beginning and ending character to identify which part of the filename contains the control identifier. The section of the filename specified will be highlighted green in the *File Name List*.



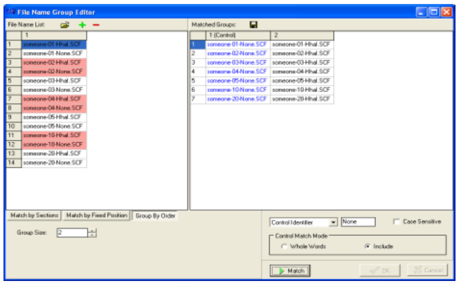
	1
1	someone-01-HhaI.SCF
2	someone-01-None.SCF
3	someone-02-HhaI.SCF
4	someone-02-None.SCF
5	someone-03-HhaI.SCF
6	someone-03-None.SCF
7	someone-04-HhaI.SCF
8	someone-04-None.SCF
9	someone-05-HhaI.SCF
10	someone-05-None.SCF
11	someone-10-HhaI.SCF
12	someone-10-None.SCF
13	someone-20-HhaI.SCF
14	someone-20-None.SCF

	1 (Control)	2
1	someone-01-None.SCF	someone-01-HhaI.SCF
2	someone-02-None.SCF	someone-02-HhaI.SCF
3	someone-03-None.SCF	someone-03-HhaI.SCF
4	someone-04-None.SCF	someone-04-HhaI.SCF
5	someone-05-None.SCF	someone-05-HhaI.SCF
6	someone-10-None.SCF	someone-10-HhaI.SCF
7	someone-20-None.SCF	someone-20-HhaI.SCF

Group Identification:
Compare Character 1-10
Control Identification:
Match to Identifier 12-14
Control Identifier N
☑ Case Sensitive
Control Match Mode
○ Whole Words ● Include

Match by Group Order

Allows the user to group samples that contain sequential identifiers.



	1
1	someone-01-HhaI.SCF
2	someone-01-None.SCF
3	someone-02-HhaI.SCF
4	someone-02-None.SCF
5	someone-03-HhaI.SCF
6	someone-03-None.SCF
7	someone-04-HhaI.SCF
8	someone-04-None.SCF
9	someone-05-HhaI.SCF
10	someone-05-None.SCF
11	someone-10-HhaI.SCF
12	someone-10-None.SCF
13	someone-20-HhaI.SCF
14	someone-20-None.SCF

	1 (Control)	2
1	someone-01-None.SCF	someone-01-HhaI.SCF
2	someone-02-None.SCF	someone-02-HhaI.SCF
3	someone-03-None.SCF	someone-03-HhaI.SCF
4	someone-04-None.SCF	someone-04-HhaI.SCF
5	someone-05-None.SCF	someone-05-HhaI.SCF
6	someone-10-None.SCF	someone-10-HhaI.SCF
7	someone-20-None.SCF	someone-20-HhaI.SCF

Group Size: 2
Control Identifier None
☐ Case Sensitive
Control Match Mode
○ Whole Words ● Include

Group Size: Enter the number of groups for the analysis. Standard MS-MLPA analysis would use 2 groups, an LOH analyses comparing one normal sample to three grades of tumor would use 4 groups.

Control Identifier

Enter the character from the *Control Identification* section (highlighted green) that describes the control or reference sample. Example: N = normal or R = reference. Select **Case Sensitive** if the *Control Identifier* needs to be identified by upper or lower case letters.

Control Match Mode

Choose either **Whole Words** or **Include**.

Whole Words should be used if the characters entered into the *Control Identifier* field need to match exactly.

Include should be selected if the characters in the *Control Identifier* field only need to be identified in the filename, i.e. not an exact match.

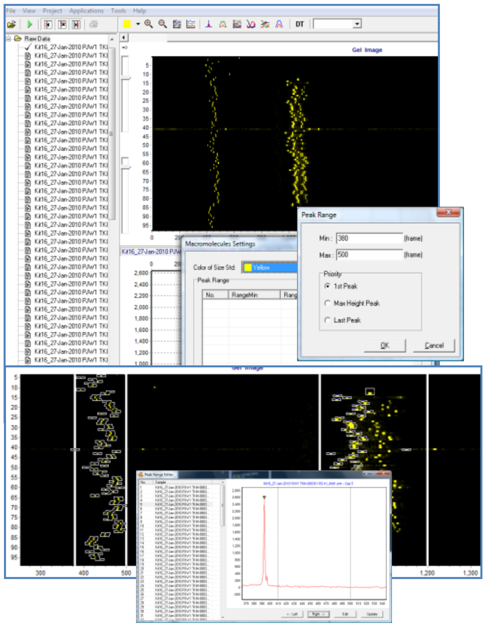
Macromolecules

Tools → Macromolecules

The Macromolecule Tool aids with analysis of macromolecules without an internal lanes size standard. Depending on the macromolecule size and configuration, migration rates through CE vary greatly. Internal size standards of the same macromolecule may not be readily available. This tool enables researchers to physically identify reference peaks known to have the same size and uses the information to calibrate from one capillary to another. Characteristics of the aligned data (such as relative size, peak height, peak area) can then be exported in an excel sheet or printed as an allele report.

Procedure

1. Select File → Import Data from the main analysis window
2. Select Tools → Macromolecules → Settings
3. Select Add and enter peak range for one group of fragments
4. Repeat for a second group of fragments at the opposite end of the size range
5. Select Ok and verify fragment selection (white boxed)
6. If needed, select Tools → Macromolecules →Range to launch the Peak Range Editor and correct for any fragments outside of the range
7. Align the results, Tools →Macromolecules → Show Results
8. Analyze data with Project → Run → Select Analysis type Animal → Next → Next → OK
9. Review data, Export Report Table (.xls or .txt) or Print the Allele Report
10. If desired, a panel may be made in the Panel Editor to allow application of local max filter in the Run Wizard
11. Project → Run → Select Panel from the drop down menu
12. Next → Next → OK

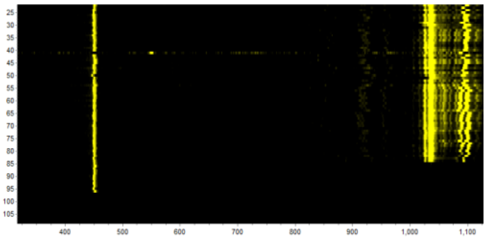


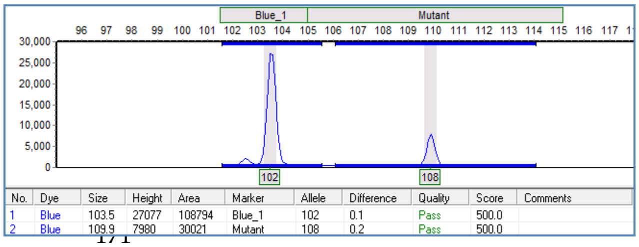
Color of Size Std: Yellow

Min : 380 (frame)

Max : 500 (frame)

● 1st Peak





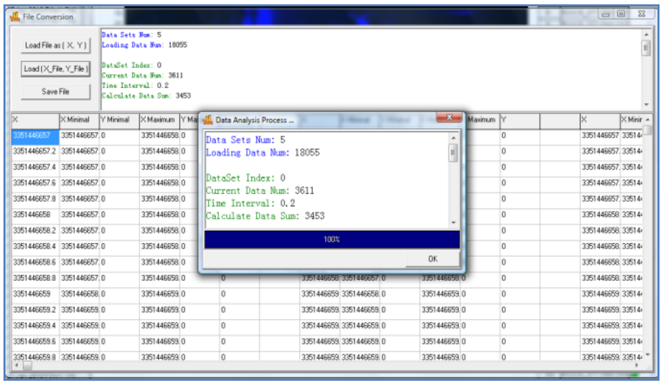
No.	Dye	Size	Height	Area	Marker	Allele	Difference	Quality	Score	Comments
1	Blue	103.5	27077	108794	Blue_1	102	0.1	Pass	500.0	
2	Blue	109.9	7980	30021	Mutant	108	0.2	Pass	500.0	

File Conversion

The file conversion tool is used by institutions developing their own fragment analysis instruments that have variable time frames for collection of data points.

Procedure

1. Tools → File Conversion to activate the File conversion screen
2. Select Load File as (X,Y) if time and peak height information is contained in the same file
3. Select Load (X_File Y_File) if the time and peak height information is contained in two files
4. Save the converted .sg1 file for import into GeneMarker



```
Data Sets Num: 5
Loading Data Num: 18055

DataSet Index: 0
Current Data Num: 3611
Time Interval: 0.2
Calculate Data Sum: 3453
```

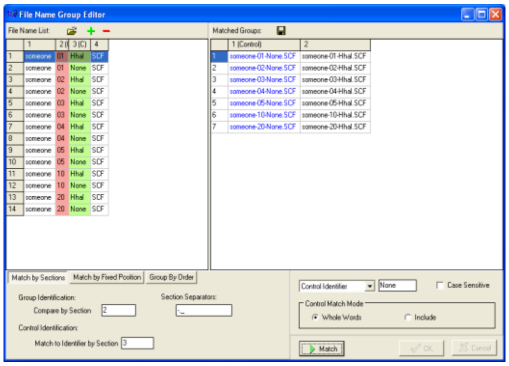
Filename Group Editor

Tools → File Name Group Tool <u>OR</u> Project → Apply Sample Grouping

The *Filename Group Editor* was originally developed to be used in conjunction with the Microsatellite Instability and Loss of Heterozygosity applications where tumor samples are compared to normal samples from the same patient. Additionally, the *Filename Group Editor* can be used in the *Main Analysis* window *Sample File Tree*.

Procedure

1. Select *Tools → File Name Group Tool <u>OR</u> Project → Apply Sample Grouping*
2. The *File Name Group Editor* window appears
3. Click the **Load Files** icon and select all files to pair
4. Choose *Match by Sections* or *Match by Fixed Position* or *Group by Order*
5. Enter values for the *Group Identification* and *Control Identification* fields
6. Enter a *Control Identifier* value and click **Match**
7. The samples from the *File Name List* will be paired into groups in the *Matched Groups* window
8. When the samples are paired correctly, click the **Save Groups to File** icon
9. Save the group information as a tab-delimited Text (.txt) file
10. Upload this filename group file into the *Group File fields* of the MSI or LOH analysis settings box <u>OR</u> the grouping will automatically be applied if *Project → Apply Sample Grouping* was chosen.



	1	2 (	3 (C)	4
1	someone	01	Hhal	SCF
2	someone	01	None	SCF
3	someone	02	Hhal	SCF
4	someone	02	None	SCF
5	someone	03	Hhal	SCF
6	someone	03	None	SCF
7	someone	04	Hhal	SCF
8	someone	04	None	SCF
9	someone	05	Hhal	SCF
10	someone	05	None	SCF
11	someone	10	Hhal	SCF
12	someone	10	None	SCF
13	someone	20	Hhal	SCF
14	someone	20	None	SCF

	1 (Control)	2
1	someone-01-None.SCF	someone-01-Hhal.SCF
2	someone-02-None.SCF	someone-02-Hhal.SCF
3	someone-03-None.SCF	someone-03-Hhal.SCF
4	someone-04-None.SCF	someone-04-Hhal.SCF
5	someone-05-None.SCF	someone-05-Hhal.SCF
6	someone-10-None.SCF	someone-10-Hhal.SCF
7	someone-20-None.SCF	someone-20-Hhal.SCF

Group Identification:
Compare by Section: 2
Section Separators: -._
Control Identification:
Match to Identifier by Section: 3
Control Identifier: None
☐ Case Sensitive
Control Match Mode: ● Whole Words ○ Include

Icons and Functions

Load Files
Opens a directory window where raw data files can be located and uploaded to the *Filename Group Editor*

Add Files
Opens a directory window where additional raw data files can be uploaded into the *Filename List* field

Remove Files

Removes any files selected in the *Filename List*. Select multiple files to remove by holding down the **SHIFT** key and selecting additional samples

Save Groups to File

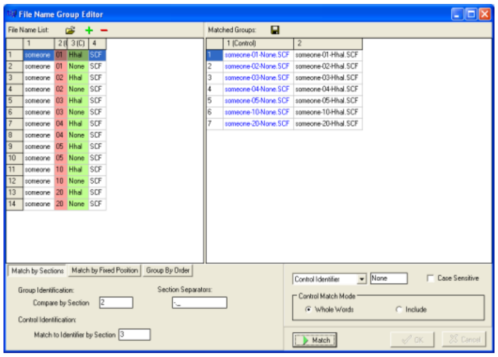
Saves the filenames of the samples paired in the *Matched Groups* field. Samples identified as *Controls* will be in the first column of the *Matched Groups* tab-delimited Text file.

Match by Sections

Automatically separates the sample filenames into groups based on the specified *Section Separators*.

Group Identification: Identifies how to match the filenames into groups based on the section entered into the *Compare by Section* field. The section of the filename specified will be highlighted red in the *File Name List*.

Control Identification: Identifies which section of the filename contains the reference vs. sample information based on the section number entered in the *Match to Identifier by Section* field. The section of the filename specified will be highlighted green in the *File Name List*.



File Name List:

	1	2 (	3 (C)	4
1	someone	01	HhaI	SCF
2	someone	01	None	SCF
3	someone	02	HhaI	SCF
4	someone	02	None	SCF
5	someone	03	HhaI	SCF
6	someone	03	None	SCF
7	someone	04	HhaI	SCF
8	someone	04	None	SCF
9	someone	05	HhaI	SCF
10	someone	05	None	SCF
11	someone	10	HhaI	SCF
12	someone	10	None	SCF
13	someone	20	HhaI	SCF
14	someone	20	None	SCF

Matched Groups:

	1 (Control)	2
1	someone-01-None.SCF	someone-01-HhaI.SCF
2	someone-02-None.SCF	someone-02-HhaI.SCF
3	someone-03-None.SCF	someone-03-HhaI.SCF
4	someone-04-None.SCF	someone-04-HhaI.SCF
5	someone-05-None.SCF	someone-05-HhaI.SCF
6	someone-10-None.SCF	someone-10-HhaI.SCF
7	someone-20-None.SCF	someone-20-HhaI.SCF

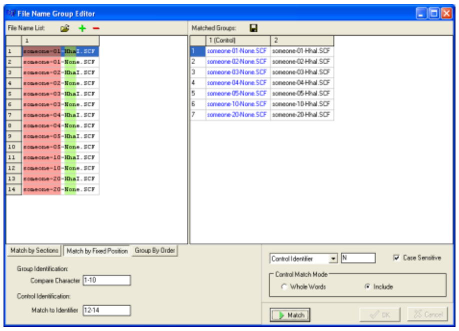
Group Identification:
Compare by Section: 2
Section Separators: -_
Control Identification:
Match to Identifier by Section: 3
Control Identifier: None
☐ Case Sensitive
Control Match Mode: ● Whole Words ○ Include

Match by Fixed Position

Allows the user to manually identify the characters of the filename for grouping the samples. *Section Separators* like "_ , -" are counted as individual characters.

Group Identification: Enter the number of the beginning and ending character to identify how to group the samples. The section of the filename specified will be highlighted red in the *File Name List*.

Control Identification: Enter the number of the beginning and ending character to identify which part of the filename contains the control identifier. The section of the filename specified will be highlighted green in the *File Name List*.



File Name List:

	1
1	someone-01-HhaI.SCF
2	someone-01-None.SCF
3	someone-02-HhaI.SCF
4	someone-02-None.SCF
5	someone-03-HhaI.SCF
6	someone-03-None.SCF
7	someone-04-HhaI.SCF
8	someone-04-None.SCF
9	someone-05-HhaI.SCF
10	someone-05-None.SCF
11	someone-10-HhaI.SCF
12	someone-10-None.SCF
13	someone-20-HhaI.SCF
14	someone-20-None.SCF

Matched Groups:

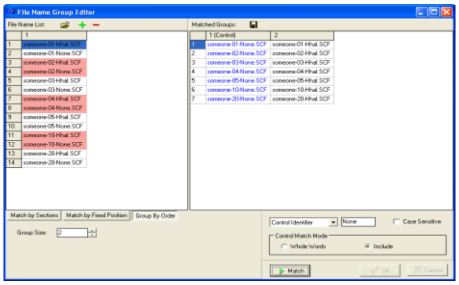
	1 (Control)	2
1	someone-01-None.SCF	someone-01-HhaI.SCF
2	someone-02-None.SCF	someone-02-HhaI.SCF
3	someone-03-None.SCF	someone-03-HhaI.SCF
4	someone-04-None.SCF	someone-04-HhaI.SCF
5	someone-05-None.SCF	someone-05-HhaI.SCF
6	someone-10-None.SCF	someone-10-HhaI.SCF
7	someone-20-None.SCF	someone-20-HhaI.SCF

Group Identification:
Compare Character: 1-10
Control Identification:
Match to Identifier: 12-14
Control Identifier: N
☑ Case Sensitive
Control Match Mode: ○ Whole Words ● Include

Match by Group Order

Allows the user to group samples that contain sequential identifiers.

Group Size: Enter the number of groups for the analysis. Standard MS-MLPA analysis would use 2 groups, an LOH analyses comparing one normal sample to three grades of tumor would use 4 groups.



File Name List:

	1
1	someone-01-HhaI.SCF
2	someone-01-None.SCF
3	someone-02-HhaI.SCF
4	someone-02-None.SCF
5	someone-03-HhaI.SCF
6	someone-03-None.SCF
7	someone-04-HhaI.SCF
8	someone-04-None.SCF
9	someone-05-HhaI.SCF
10	someone-05-None.SCF
11	someone-10-HhaI.SCF
12	someone-10-None.SCF
13	someone-20-HhaI.SCF
14	someone-20-None.SCF

Matched Groups:

	1 (Control)	2
1	someone-01-None.SCF	someone-01-HhaI.SCF
2	someone-02-None.SCF	someone-02-HhaI.SCF
3	someone-03-None.SCF	someone-03-HhaI.SCF
4	someone-04-None.SCF	someone-04-HhaI.SCF
5	someone-05-None.SCF	someone-05-HhaI.SCF
6	someone-10-None.SCF	someone-10-HhaI.SCF
7	someone-20-None.SCF	someone-20-HhaI.SCF

Group Size: 2
Control Identifier: None
☐ Case Sensitive
Control Match Mode: ○ Whole Words ● Include

Control Identifier

Enter the character from the *Control Identification* section (highlighted green) that describes the control or reference sample. Example: N = normal or R = reference. Select **Case Sensitive** if the *Control Identifier* needs to be identified by upper or lower case letters.

Control Match Mode

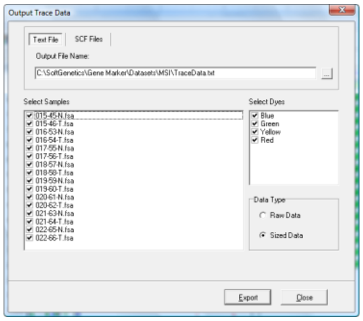
Choose either **Whole Words** or **Include**.
Whole Words should be used if the characters entered into the *Control Identifier* field need to match exactly.

Include should be selected if the characters in the *Control Identifier* field only need to be identified in the filename, i.e. not an exact match.

Output Trace Data

Tools → Output Trace Data

The *Output Trace Data* tool exports raw or sized data of uploaded sample files as Text (*.txt) or SCF (*.scf) or SG1 (*.sg1)files.



Output File Name: C:\SoftGenetics\Gene Marker\Datasets\MSI\TraceData.txt

Select Samples:
- ☑ 015-45-N.fsa
- ☑ 015-46-T.fsa
- ☑ 016-53-N.fsa
- ☑ 016-54-T.fsa
- ☑ 017-55-N.fsa
- ☑ 017-56-T.fsa
- ☑ 018-57-N.fsa
- ☑ 018-58-T.fsa
- ☑ 019-59-N.fsa
- ☑ 019-60-T.fsa
- ☑ 020-61-N.fsa
- ☑ 020-62-T.fsa
- ☑ 021-63-N.fsa
- ☑ 021-64-T.fsa
- ☑ 022-65-N.fsa
- ☑ 022-66-T.fsa

Select Dyes:
- ☑ Blue
- ☑ Green
- ☑ Yellow
- ☑ Red

Data Type:
- ○ Raw Data
- ● Sized Data

Procedure

1. Select whether to export the data as a *Text* or *SCF* file
2. Choose the directory and folder to save the exported data to in the *Output File Name* field.
3. Select the samples to include in the output file from the *Select Samples* field.
4. Select which dye color data to export from the *Select Dyes* field.
5. Select whether to export raw or sized data from the *Data Type* options.
6. Click **Export** to export the data to the specified folder.

Project Comparison

Tools → Project Comparison

The *Project Comparison* tool can serve two functions. First, it can be used to compare two independent analysts' analyses. Second, it can be used as a validation tool to determine differences in allele calls based on analysis parameters or instrument runs.

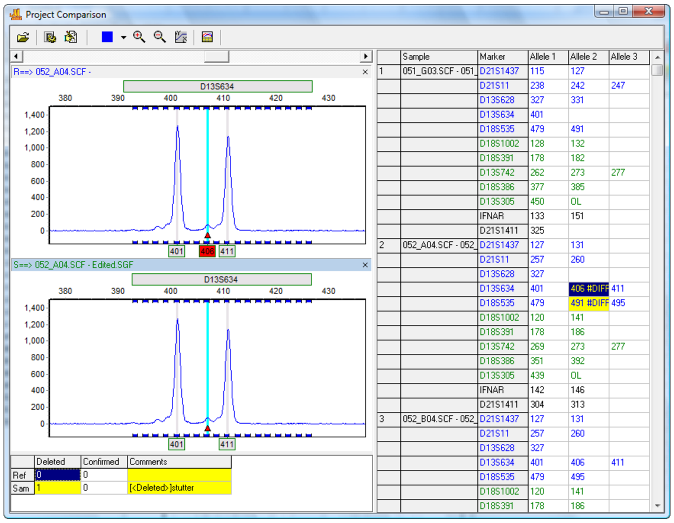
Procedure

1. After initial dataset analysis, select *Tools → Project Comparison*
2. The *Project Comparison* window appears
3. Click the **Open Project to Compare** icon
4. Use the file directory window to locate and select a previously saved SoftGenetics project file (.sgf, .sfp)

NOTE: Projects with similar datasets and analysis types should be chosen.

5. Click **Open** and the second project will be uploaded to the *Project Comparison* tool
6. The first project originally loaded into GeneMarker will be marked as the ***Reference (R=>)*** and the second project uploaded to the *Project Comparison* tool is marked as the ***Sample (S=>)***
7. Click the **Project Comparison Settings** icon to choose parameters to compare between the projects
8. Differences will be indicated in the report table on the right. When a difference is selected, each project's electropherogram and peak table will be displayed on the left.

Project Comparison Tool



	Deleted	Confirmed	Comments
Ref	0	0	
Sam	1	0	[<Deleted>]stutter

	Sample	Marker	Allele 1	Allele 2	Allele 3
1	051_G03.SCF - 051_	D21S1437	115	127	
		D21S11	238	242	247
		D13S628	327	331	
		D13S634	401		
		D18S535	479	491	
		D18S1002	128	132	
		D18S391	178	182	
		D13S742	262	273	277
		D18S386	377	385	
		D13S305	450	OL	
		IFNAR	133	151	
		D21S1411	325		
2	052_A04.SCF - 052_	D21S1437	127	131	
		D21S11	257	260	
		D13S628	327		
		D13S634	401	406 #DIFF	411
		D18S535	479	491 #DIFF	495
		D18S1002	120	141	
		D18S391	178	186	
		D13S742	269	273	277
		D18S386	351	392	
		D13S305	439	OL	
		IFNAR	142	146	
		D21S1411	304	313	
3	052_B04.SCF - 052_	D21S1437	127	131	
		D21S11	257	260	
		D13S628	327		
		D13S634	401	406	411
		D18S535	479	495	
		D18S1002	120	141	
		D18S391	178	186	

Icons and Functions

Open Project to Compare

Opens a directory window for the user to identify a similar project to compare to the project already running in GeneMarker. The first project in GeneMarker will be considered the Reference project and the project uploaded to the *Project Comparison* tool will be considered the Sample project

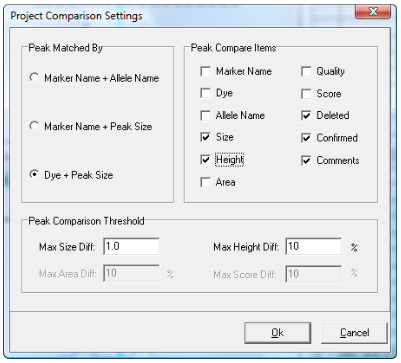
Project Comparison Settings

Launches the *Project Comparison Settings* box with several options for running the comparison.

Peak Matched By: Allows the user to choose the principal parameters for comparison.

Peak Compare Items: Options for which parameters should be compared and marked as different.

Peak Comparison Threshold: Allows the user to qualify the ranges for detecting differences in peak attributes.


Project Comparison Settings

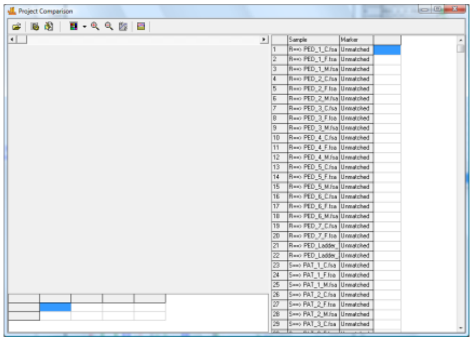
Peak Matched By
○ Marker Name + Allele Name
○ Marker Name + Peak Size
● Dye + Peak Size

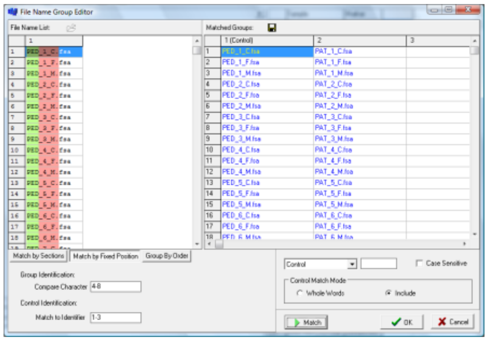
Peak Compare Items
☐ Marker Name
☐ Dye
☐ Allele Name
☑ Size
☑ Height
☐ Area
☐ Quality
☐ Score
☑ Deleted
☑ Confirmed
☑ Comments

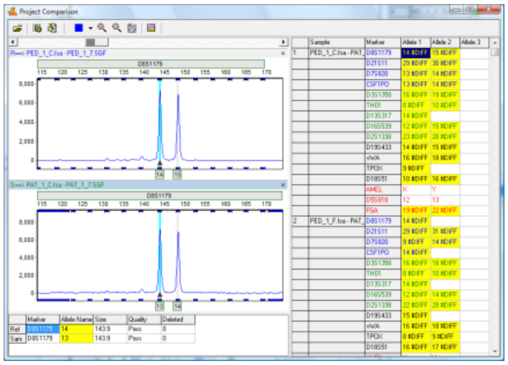
Peak Comparison Threshold
Max Size Diff: 1.0
Max Height Diff: 10 %
Max Area Diff: 10 %
Max Score Diff: 10 %

File Grouping Tool

Provides the ability to group duplicate files with different file names analyzed from different genetic analyzers. . If the projects have different file names they will appear in a list at the right side of the screen after selecting in the open folder process described above. Use the file group tool > match by section or fixed position > 'Match' > 'OK'







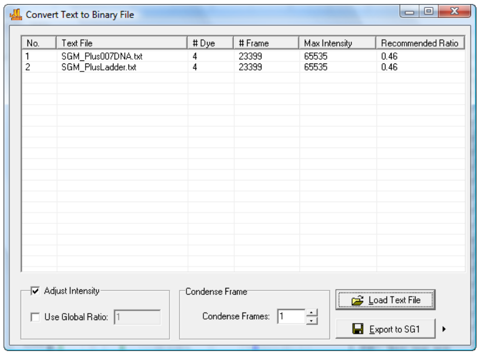
Convert TXT to Binary

Tools → Convert Text to Binary Files

The *Convert Text to Binary* tool allows the user to upload trace data information in Text (.txt) file format for conversion into a four-color SCF file or a five-color SG1 file. The SCF and SG1 files can then be read by GeneMarker and translated into chromatograms. This tool is useful for institutions developing their own fragment analysis instruments.

Procedure

1. Click the **Load Text File** button and select Text (.txt) files to convert
2. Once files are uploaded, they will appear in the *Text File* field
3. The software will automatically calculate a *Recommended Ratio* for the user to condense the number of frames in a single trace
4. Enter a condense frames by XX number in the *Condense Frames* field
5. Click **Export to SG1** if exporting a five-color trace, click **Export to SCF** if exporting a four-color trace.



No.	Text File	# Dye	# Frame	Max Intensity	Recommended Ratio
1	SGM_Plus007DNA.txt	4	23399	65535	0.46
2	SGM_PlusLadder.txt	4	23399	65535	0.46

☑ Adjust Intensity
☐ Use Global Ratio: 1
Condense Frame
Condense Frames: 1

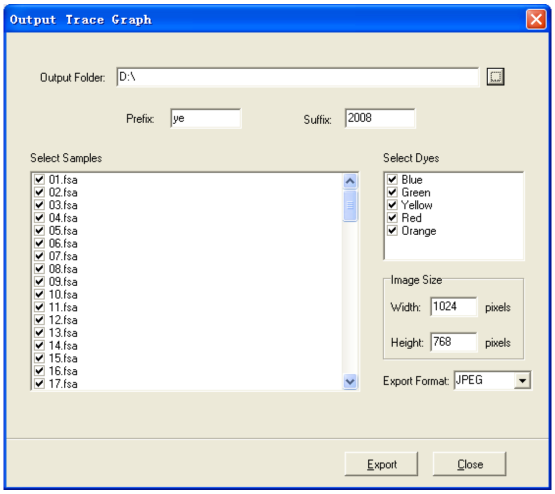
Export Elecropherogram

Tools →Export Electropherogram

The *Export Electropherogram* tool allows the user to export the trace images to a specified folder.

Procedure

1. Use a dropdown menu to specify the output folder.
2. Specify the prefix and suffix for the exported file name. The full file name will be Prefix+Sample name+”_”+Dye name+Suffix+Extension name.
3. Select samples, Dyes and Image Size
4. Use a dropdown menu to specify the export format. JPEG and PNG are both available. PNG is recommended



Output Folder: D:\
Prefix: ye
Suffix: 2008

Select Samples
☑ 01.fsa
☑ 02.fsa
☑ 03.fsa
☑ 04.fsa
☑ 05.fsa
☑ 06.fsa
☑ 07.fsa
☑ 08.fsa
☑ 09.fsa
☑ 10.fsa
☑ 11.fsa
☑ 12.fsa
☑ 13.fsa
☑ 14.fsa
☑ 15.fsa
☑ 16.fsa
☑ 17.fsa

Select Dyes
☑ Blue
☑ Green
☑ Yellow
☑ Red
☑ Orange

Image Size
Width: 1024 pixels
Height: 768 pixels
Export Format: JPEG

Chapter 9 User Management

Chapter 9 User Management

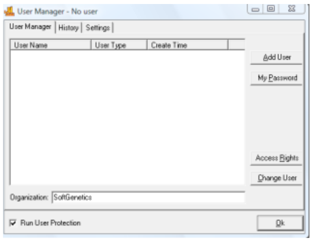
Procedure
User Manager
History
Settings
Edit History/Audit Trail

Overview

User management may be implemented after installation of GeneMarker. The administrator activates User Managerment from the Help drop-down menu. User management provides control of user access rights, automatically generates an audit trail of all edits to genotyping projects and is used to customize the Organization and Operator fields in final print reports of special applications such as MLPA and Trisomy.

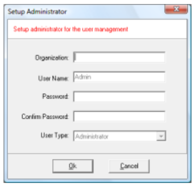
Procedure

1. Select *Help* → *User Management*
2. The *Login* box appears
3. Click Run User Protection to activate the setup Administrator
4. Enter Organization Name, an Administrator username and password
5. Click **OK**
6. You are now logged in as the *Administrator*
7. Click the **Add User** button to add additional users
8. Click the **Access Rights** button to set up user type access permissions
9. Be sure to select *Run User Protection* and click **OK** to exit



Organization: SoftGenetics

☑ Run User Protection



Setup administrator for the user management

Organization:

User Name: Admin

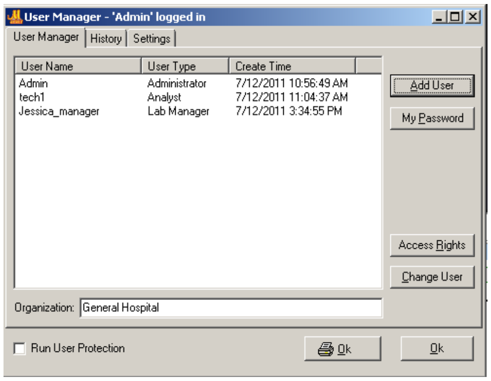
Password:

Confirm Password:

User Type: Administrator

User Manager

The *User Manager* tab displays user information and contains options for creating and deleting users.



User Name	User Type	Create Time
Admin	Administrator	7/12/2011 10:56:49 AM
tech1	Analyst	7/12/2011 11:04:37 AM
Jessica_manager	Lab Manager	7/12/2011 3:34:55 PM

Organization: General Hospital

☐ Run User Protection

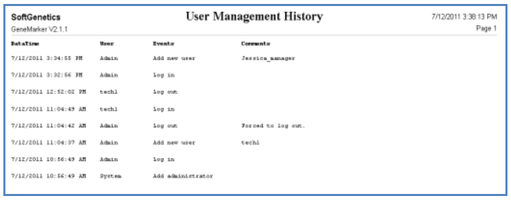
User Window
Displays all users by name, type and creation date

Organization
Enter your organization name

Run User Protection
When selected, users will be prompted to log on with a user name and password. When deselected, any person can launch GeneMarker without a username and password.

Add User
Launches the *Add User* box where a new username and password can be input. This is also where the user type can be chosen. A user can be deleted by right-clicking the username and selecting *Delete User*.
NOTE: Only the *Administrator* can add and delete users.

Print Icon
Launches the print preview to print the user management history or save as a .pdf document.



SoftGenetics
GeneMarker V2.1.1

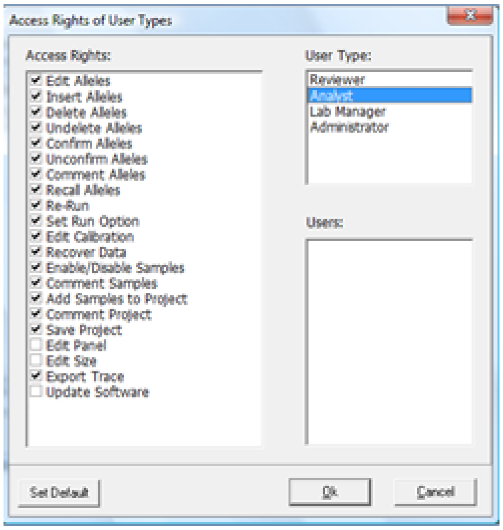
User Management History

7/12/2011 3:38:13 PM
Page 1

DateTime	User	Events	Comments
7/12/2011 3:34:55 PM	Admin	Add new user	Jessica_manager
7/12/2011 3:32:56 PM	Admin	Log in	
7/12/2011 12:52:02 PM	tech1	Log out	
7/12/2011 11:04:49 AM	tech1	Log in	
7/12/2011 11:04:42 AM	Admin	Log out	Forced to log out.
7/12/2011 11:04:37 AM	Admin	Add new user	tech1
7/12/2011 10:56:49 AM	Admin	Log in	
7/12/2011 10:56:49 AM	System	Add administrator	

My Password
Launches the *Change Password* box where the user that is logged in can enter a new password. The new password must be entered twice to ensure accuracy.

Access Rights
Launches the *Access Rights of User Types* box where the different access rights available to each user type can be identified. Clicking the **Set Default** button will return the *Access Rights* for the *User Type* selected back to factory defaults.
NOTE: Only the *Administrator* can change *Access Rights* for a *User Type*.



Access Rights:
☑ Edit Alleles
☑ Insert Alleles
☑ Delete Alleles
☑ Undelete Alleles
☑ Confirm Alleles
☑ Unconfirm Alleles
☑ Comment Alleles
☑ Recall Alleles
☑ Re-Run
☑ Set Run Option
☑ Edit Calibration
☑ Recover Data
☑ Enable/Disable Samples
☑ Comment Samples
☑ Add Samples to Project
☑ Comment Project
☑ Save Project
☐ Edit Panel
☐ Edit Size
☑ Export Trace
☐ Update Software

User Type:
Reviewer
Analyst
Lab Manager
Administrator

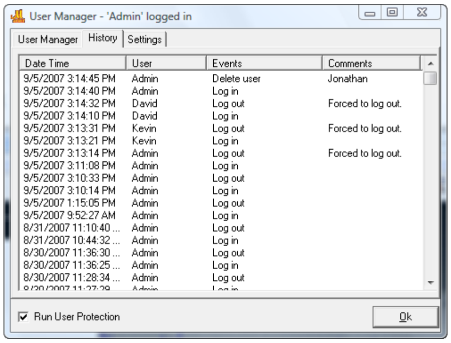
Users:

Change User

Prompts for a confirmation of action then launches the *Login* box. Choose a new user and enter the user's password to login.

History

The *User Manager History* tab monitors user activity associated with the user manager function.



Date Time	User	Events	Comments
9/5/2007 3:14:45 PM	Admin	Delete user	Jonathan
9/5/2007 3:14:40 PM	Admin	Log in	
9/5/2007 3:14:32 PM	David	Log out	Forced to log out.
9/5/2007 3:14:10 PM	David	Log in	
9/5/2007 3:13:31 PM	Kevin	Log out	Forced to log out.
9/5/2007 3:13:21 PM	Kevin	Log in	
9/5/2007 3:13:14 PM	Admin	Log out	Forced to log out.
9/5/2007 3:11:08 PM	Admin	Log in	
9/5/2007 3:10:33 PM	Admin	Log out	
9/5/2007 3:10:14 PM	Admin	Log in	
9/5/2007 1:15:05 PM	Admin	Log out	
9/5/2007 9:52:27 AM	Admin	Log in	
8/31/2007 11:10:40 ...	Admin	Log out	
8/31/2007 10:44:32 ...	Admin	Log in	
8/30/2007 11:36:30 ...	Admin	Log out	
8/30/2007 11:36:25 ...	Admin	Log in	
8/30/2007 11:28:34 ...	Admin	Log out	

☑ Run User Protection

Date/Time
Records the computer's date and time for the activity.

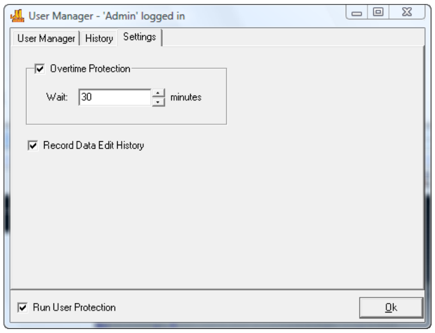
User
Identifies the username of the person that performed the activity.

Events
Records the user manager activity that was performed.

Comments
Gives additional information for the event that was performed. For example, if a user is added, then the username of the person that was added is recorded under *Comments*.

Settings

The *User Manager Settings* tab contains additional options for the *User Management* function.



☑ Overtime Protection

Wait: 30 minutes

☑ Record Data Edit History

☑ Run User Protection

Overtime Protection
When selected, GeneMarker will logout the user after the specified time entered in the *Wait* field. When the user is logged out, the status of the analysis remains unchanged until the user logs back in (with username and password).

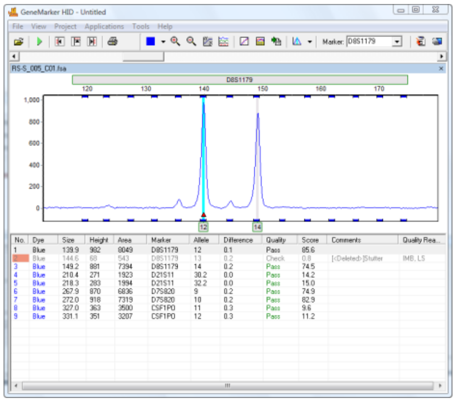
Record Data Edit History
When selected, any changes made to the allele calls of the project will be saved in the *Edit History* log. Please see *Edit History* section below for more information.

Edit History/Audit Trail

When **Record Data Edit History** is selected in the *User Manager Settings* box (see *User Management Settings* section above), any change to allele calls in the analysis will be recorded. Changes can also be recovered in the *Edit History* feature.

Procedure

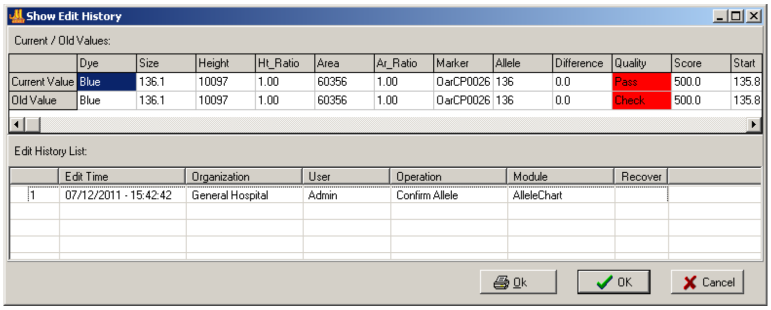
1. Click the **Show Chart/Table** icon in the *Main Analysis* window.
2. The *Peak Table* will appear below the sample electropherogram.
3. Make changes to allele calls by right-clicking any cell in that allele's row in the *Peak Table* or right-click the grey vertical bar at the center of the peak in the electropherogram.
4. Choose to *Edit Allele*, *Edit Comments*, *Add/Delete Allele*, and *Confirm*. **See Chapter 3 Main Analysis Overview**.
5. Once a change has been made to the allele call, notice the pink shading in the *No.* column of the *Peak Table*. This indicates a change has been made to that allele.
6. Right-click any changed allele and select *View History*.
7. The *Show Edit History* window appears.



No.	Dye	Size	Height	Area	Marker	Allele	Difference	Quality	Score	Comments	Quality Rea...
1	Blue	139.9	982	8049	D8S1179	12	0.1	Pass	85.6		
2	Blue	144.6	68	543	D8S1179	13	0.2	Check	0.8	[<Deleted>]Stutter	IMB, LS
3	Blue	149.2	881	7394	D8S1179	14	0.2	Pass	74.5		
4	Blue	210.4	271	1923	D21S11	30.2	0.0	Pass	14.2		
5	Blue	218.3	283	1994	D21S11	32.2	0.0	Pass	15.0		
6	Blue	267.9	870	6836	D7S820	9	0.2	Pass	74.9		
7	Blue	272.0	918	7319	D7S820	10	0.2	Pass	82.9		
8	Blue	327.0	363	3500	CSF1PO	11	0.3	Pass	9.6		
9	Blue	331.1	351	3207	CSF1PO	12	0.3	Pass	11.2		

8. Select a change from the *Edit History List* to view changes in the *Current/Old Values* table. Changes will be highlighted in red.
9. To recover a change, right-click the row in the *Edit History List* and select **Recover Old Value**. A star will appear in the *Recover* column.
10. Click **OK** and click **Yes** when the warning prompts you to confirm.
11. Click the printer icon for a print preview and print or save as .pdf options

Edits History Window



Current / Old Values:

	Dye	Size	Height	Ht_Ratio	Area	Ar_Ratio	Marker	Allele	Difference	Quality	Score	Start
Current Value	Blue	136.1	10097	1.00	60356	1.00	OarCP0026	136	0.0	Pass	500.0	135.8
Old Value	Blue	136.1	10097	1.00	60356	1.00	OarCP0026	136	0.0	Check	500.0	135.8

Edit History List:

	Edit Time	Organization	User	Operation	Module	Recover
1	07/12/2011 - 15:42:42	General Hospital	Admin	Confirm Allele	AlleleChart	

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